HISTORY, DEVELOPMENT AND SCOPE OF MICROBIOLOGY

The word "microbiology" was derived from three Greek words: *mikros* (meaning 'small'), *bios* (meaning 'life') and *logia* (meaning 'science or study'). Microbiology is the study of microorganisms or microbes, which are too small to be seen by the naked (unaided) eyes. The group includes bacteria, fungi (yeasts and molds), protozoa, and algae. It also includes viruses, those non-cellular entities sometimes regarded as straddling the border between life and nonlife. Note however that some organisms studied in this field are visible to the eyes without a microscope such as *Epulopiscium fishelsoni* (bacteria), *Thiomargarita namibiensis* (bacteria), mushroom (fungi), mould (fungi)

It is all about the study of microbial cells, how they work and affect man, plant, animals and the environment.

Discovery of Microorganisms

The invention of the microscope enabled the study of microorganisms. The first microscopes were simple ground glass lenses that magnified images of the tiny life. Among the first to observe this previously unseen and invisible microbial world were Robert Hooke and Anthony Van Leeuwenhoek.

1. **Robert Hooke (1635-1703)**, an English mathematician and natural historian was the first to coin the term "cells" to describe the "little boxes" he observed while examining cork slices with a compound microscope. He was also the first to make a known description of microorganisms.

2. Anthony Van Leeuwenhoek (1632-1723) was a Dutch draper and an amateur microscope builder. He learned lens grinding as a hobby and made over 100 simple microscopes each capable of magnifying an image about 300 times.

By using simple microscopes, he observed microscopic organisms which he called "animacules". He discovered bacteria in 1676 while studying pepper water infusion and reported his observations in a series of letters to Royal Society of London which published them in 1684 in English translation. He made sketches of the different shapes of bacteria. He was the first person to publish extensive and accurate observations of microorganisms. He is regarded as the father of microbiology.

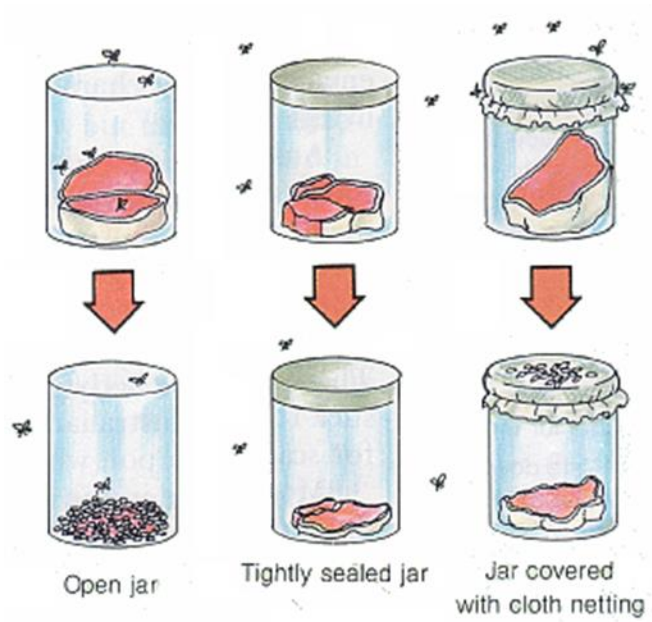
After Van Leeuwenhoek's death, the study of microbiology did not develop rapidly because microscopes were rare and interest in microorganisms was not high. Scientists then were debating the theory of spontaneous generation.

The Spontaneous Generation (Abiogenesis) Conflict

The concept spontaneous generation states that living organisms could develop from non-living matter. The proponents of the concept of spontaneous generation claim that living organisms could develop from non-living or decomposing matter.

1. Francesco Redi (1626-1697) disputed this theory by demonstrating with experiment that maggots found on decaying meat originated from eggs laid by flies on the meat, and not from the meat itself.

Francesco Redi placed fresh meat in open containers. As expected, the rotting meat attracted flies, and the meat was soon swarming with maggots, which hatched into flies. When the jars were tightly covered so that flies could not get in, no maggots were produced. To answer the objection that the cover cut off fresh air necessary for spontaneous generation, Redi covered the jars with several layers of porous gauze instead of an air-tight cover. Flies were attracted to the smell of the rotting meat, clustered on the gauze, which was soon swarming with maggots, but the meat itself remained free of maggots. Thus, this shows that flies are necessary to produce flies: they do not arise spontaneously from rotting meat.



Redi’s experiment

2. Louis Jablot (1670) conducted an experiment in which he divided a hay infusion that had been boiled into two containers: a heated container that was closed to the air and a heated container that was freely open to the air. Only the open vessel developed microorganisms. This further helped to disprove abiogenesis.

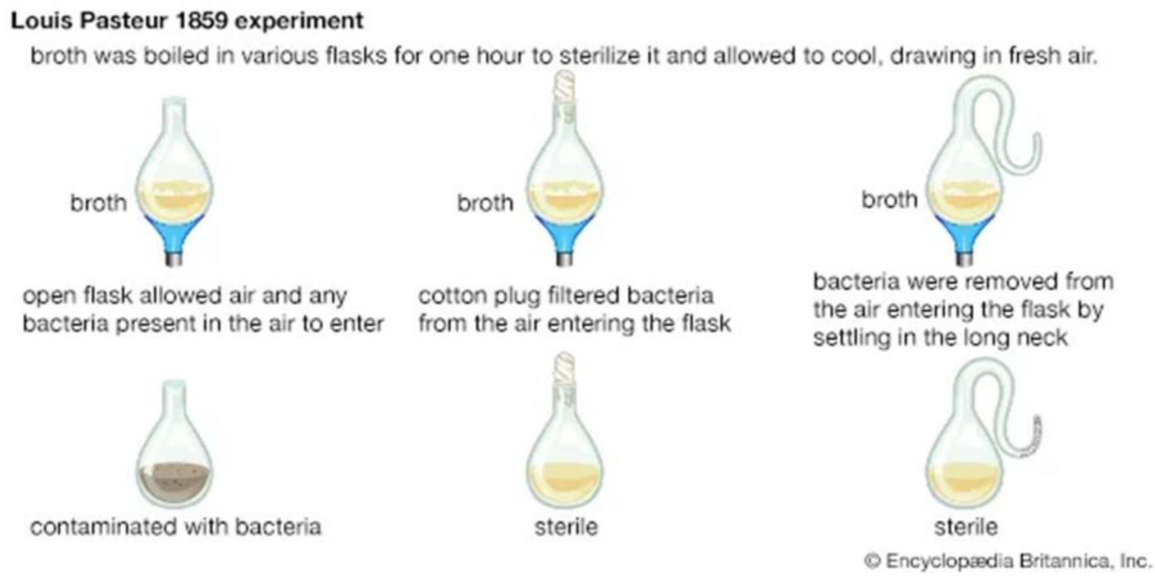
3. John Needham (1713-1781) showed that mutton broth boiled in flasks and then sealed could still develop microorganisms, which supported the theory of spontaneous generation.

4. Lazzaro Spallanzani (1729-1799) showed that flasks sealed and then boiled had no growth of microorganisms, and he proposed that air carried germs to the culture medium. He also commented that external air might be needed to support the growth of animals already in the medium. The latter concept was appealing to supporters of spontaneous generation.

5. Louis Pasteur (1822-1895) was a Professor of Chemistry. He devised series of swan necked flasks known as Pasteur-flasks, filled the flasks with broth and heated the broth to sterilization.

Pasteur became a powerful opponent of spontaneous generation. He predicted that microorganisms in putrefying materials were descendants of cells that entered from the air or cells that had initially been present on the decaying materials. Pasteur reasoned that if food were treated in such a way as to destroy all living organisms present—that is, if it were rendered sterile—and if it were kept sterile, it would not putrefy.

Pasteur used heat to kill contaminating microorganisms, and he found that extensive heating of a nutrient solution followed by sealing kept it from putrefying. Proponents of spontaneous generation criticized these experiments by declaring that “fresh air” was necessary for the phenomenon to occur. In 1864 Pasteur countered this objection simply and brilliantly by constructing the swan-necked flask, now called a *Pasteur flask*. In such a flask, nutrient solutions could be heated to boiling and sterilized. After the flask cooled, air could reenter, but the bend in the neck prevented particulate matter (including microorganisms) from entering the nutrient solution and initiating putrefaction. Nutrient solutions in such flasks remained sterile indefinitely. Microbial growth was observed only after particulate matter from the neck of the flask was allowed to enter the liquid in the flask. This experiment settled the spontaneous generation controversy forever.



Pasteur’s experiment

Apart from the defeat of the concept of spontaneous generation,

- Pasteur’s work led to an effective sterilization method which involve holding juices and milk at 62.8^{O}C for 30 minutes known as Pasteurization.
- He discovered that alcoholic fermentation was catalyzed by yeast cells.
- He developed vaccines for the disease anthrax, fowl cholera and rabies between 1880 and 1890.
- He postulated the Germ Theory of Disease which states that microorganisms are the cause of infectious diseases.
- Pasteur’s works ushered in the Golden Age of Microbiology.

The Recognition of the Role of Microorganisms in Disease

1. Agostino Bassi (1773-1856) showed that silkworm disease was caused by a fungus.

2. M. J. Berkerley (ca. 1845) demonstrated that the great potato blight of Ireland was caused by a fungus.

3. Joseph Lister (1872-1912) developed a system of surgery designed to prevent microorganisms from entering wounds. He implemented the use of sterile surgical instrument, and used carbolic acid (phenol) during surgery and on wound dressings.

4. Robert Koch (1843-1910) was a German physician. He was the first to directly prove the role of microorganisms in causing diseases. He established the relationship between *Bacillus anthracis* and the disease it causes, anthrax. Using mice as experimental animals, he demonstrated that when a small amount of blood from a diseased mouse was injected into a healthy mouse, the healthy mouse quickly developed anthrax. From this work he developed Koch's postulates.

Koch's postulates are:

- The suspected disease-causing organism should be present in all cases of the disease and absent from healthy animals.
- The suspected organism must be cultivated in a pure culture away from the animal body.
- The isolated organism must cause the disease when inoculated into a healthy susceptible animal.
- The organism must be re-isolated from these experimental animals and culture again in the laboratory after which it should still be the same as the original organism.

Using these principles, Koch discovered causative organisms of anthrax (1876), tuberculosis (1882) and cholera (1883).

- He was also the first to grow bacteria on solid culture media to get pure culture; hence he developed the pure culture concept and developed different solid media.
- Koch's discovery of solid culture media and pure culture concept supplied the most needed tools for the development of microbiology as a field of science.
- For his contribution on tuberculosis, he was awarded the 1905 Nobel Prize for Physiology or Medicine. Today, "Molecular Koch's postulates" have been established in light of advances in the molecular biology of pathogenic microbes.

5. Edward Jenner (ca. 1798) used a vaccination procedure to protect individuals from smallpox.

6. Emil von Behring (1854-1917) and **Shibasaburo Kitasato (1852-1931)** induced the formation of diphtheria tetanus antitoxins in rabbits which were effectively used to treat humans, thus demonstrating humoral immunity.

The Discovery of Microbial Effects on Organic and Inorganic Matter

1. Martinus Beijerinck (1851-1931) isolated the first pure culture of many soil and aquatic microorganisms, including sulphate reducing and sulfur oxidizing bacteria, nitrogen fixing root nodule bacteria. He also described the first virus and the basic principles of virology. He is regarded as the Father of virology and one of the founders of environmental microbiology.

2. Sergei Winogradsky (1856-1953) proposed the concept of Chemolithotrophy (the oxidation of inorganic matter). He worked with soil bacteria and discovered they could oxidize iron, sulphur and ammonia to obtain energy. He also studied anaerobic nitrogen fixation and cellulose decomposition.

Beijerinck and Winogradsky pioneered the use of enrichment cultures and selective media.

The Development of Microbiology in the 20th Century

Microbiology established a closer relationship with other disciplines during the 1940s because of its association with genetics and biochemistry.

1. George W. Beadle and Edward L. Tatum (ca. 1941) studied the relationship between genes and enzymes using the bread mould, Neurospora.

2. Salvadore Lurai and Max Delbruck (ca. 1943) showed that mutations were spontaneous and not directed by the environment.

3. Oswald T. Avery, Colin M. Mcleod, and Maclyn McCarty (1944) presented evidence that showed deoxyribonucleic acid (DNA) was the genetic material and carried genetic information during transformation.

Era of Molecular Microbiology began in the 1950s.

- Advancement in the knowledge of bacterial physiology, biochemistry and genetics.

- Genetic manipulation which involves the transfer of DNA from one organism into another or a bacterium and the proteins encoded by the DNA harvested led to the development of the field of Biotechnology.

- DNA sequencing revealed the phylogenetic (evolutionary) relationships among bacteria which led to revolutionary new concepts in microbial systematic.

- In the 1990s, DNA sequencing gave birth to the field of genomics.

The Scope of Microbiology

Microbiology as a field has two main branches:

1. Basic

2. Applied

Both branches intertwine and are complementary to each other. The basic branch of microbiology is concerned with the study of the biology of microorganisms. Fields of study here include:

1. Bacteriology: This is the study of bacteria.

2. Mycology: The study of fungi such as yeasts, molds, and mushrooms.

3. Phycology or Algology: The study of algae.

4. Protozoology: The study of protozoa. A branch of protozoology called parasitology deals exclusively with the parasite or disease producing protozoa and other parasitic micro and macro organisms.

5. Microbial Cytology: Studies the structures of microbial cells.

6. Microbial Physiology: Studies of the nutrients that microorganisms require for metabolism, growth and the products that they make from nutrients.

7. Microbial Genetics: Focuses on the nature of genetic information in microorganisms and how it regulates the development and functions of cells and organisms.

8. Microbial Ecology: The study of microorganisms in their natural environment. It also studies the global and local contribution to nutrient cycling.

9. Microbial Taxonomy: This is the study of the classification of microorganisms or the grouping of microorganisms.

10. Virology: This deals with the study of viruses and virus-like agents

The applied aspect of microbiology deal with practical application of microorganisms to solve problems related to diseases, water and waste water treatment, food spoilage and food production. The various fields of study in applied microbiology include:

1. Medical Microbiology: Studies of the causative agents of diseases, diagnostic procedures for identification of the causative agents and preventive measures.

2. Agricultural Microbiology: This is the study of microbial processes in the soil to promote plant growth. It involves the study of soil microorganisms which has led to the discovery of antibiotics and other important chemicals. It also deals with the methods of combating plant and animal diseases caused by microbes, methods of using microbes to increase soil fertility and crop yields. Currently, much work is being done on using bacterial and viral insect pathogens to substitute chemical pesticides.

3. Industrial Microbiology: This is the large-scale growth of microorganisms for the production of medicinal products such as antibiotics and vaccines, fermented beverages, industrial chemicals, production of hormones and proteins by genetically engineered microorganism.

4. Aquatic and Marine Microbiology: Aquatic and Marine Microbiology deals with microbial processes in lakes, rivers, and the oceans. It also examines issues that concern water purification, microbiology examination and biological degradation of waste.

5. Public Health Microbiology: This is closely related to medical microbiology. It deals with the identification and the control of the spread of communicable diseases. It involves monitoring of community food establishments and waste supplies so as to keep them safe and free from infectious agents.

6. Immunology: Deals with how the immune system protects the body from pathogens and the response of infectious agents. It also involves practical health problem such as the nature and treatment of allergies auto-immune diseases.

7. Food and Diary Microbiology: Involves with the use of microbes to make foods such as cheese, yoghurt, wine and beer. It also deals with the methods of preventing microbial spoilage of food and the transmission of food-borne diseases such as Botulism and Salmonellosis. Microorganisms are also used as single cell protein, which is an important source of protein or nutrients to livestock and humans.

8. Aeromicrobiology: Advances thought in the dissemination of diseases in the air, contamination and spoilage.

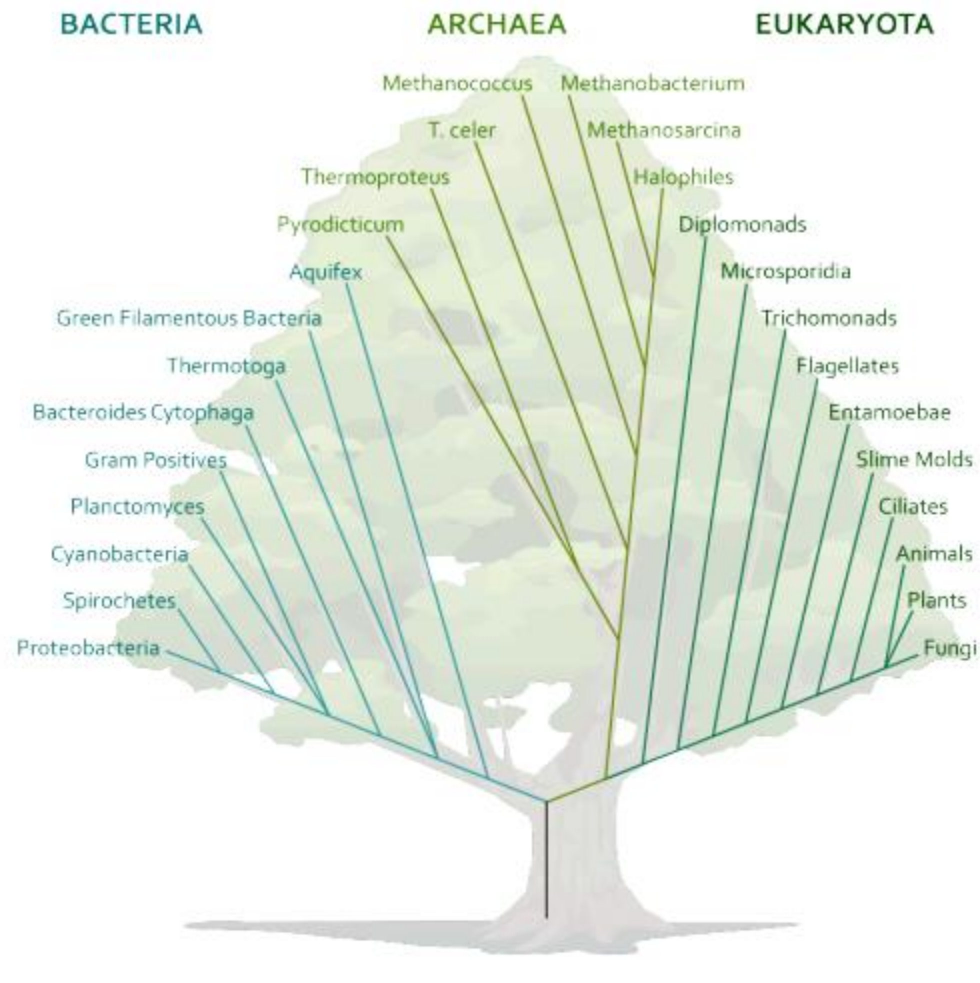
9. Astromicrobiology or Exomicrobiology: Exploration for life in outer space.

10. Geochemical Microbiology: the study of the interactions between microorganisms and the chemical components of the Earth's environment, including the atmosphere, hydrosphere, lithosphere, and biosphere.

Microbiology has made substantial contribution to scientific advancements, as evidenced by the significant representation of microbiologists and investigators using microbial models among Nobel Prize winners. This underscores the pivotal role of microbiology in shaping our understanding of fundamental biological processes and its impact on various fields such as medicine, agriculture, and biotechnology.

CHARACTERISTICS OF MICROBIAL GROUPS

The classification of organisms, or determination of how they are grouped, continually changes as new information and new tools of assessing the characteristics of an organism are acquired. Currently all organisms are grouped into one of three categories or domains: **Bacteria**, **Archaea**, and **Eukarya**. The three Domain Classification, first proposed by Carl Woese in the 1970s, is based on ribosomal RNA (rRNA) sequences and widely accepted by scientists today as the most accurate current portrayal of how organism are related.



The phylogenic Tree of Life

Source: General Microbiology by Linda Bruslind

Bacteria

The Bacteria domain contains some of the best-known microbes *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*. Most of the members are unicellular, cells lack a nucleus or any other organelle, most members have a cell wall with a particular substance known as peptidoglycan (which is only found in bacteria), they have 70S ribosomes (“S” being unit of measurement called Svedberg). They are common in soil, water, our foods, and our own bodies. All Bacteria are considered microbes with a few exceptions.

Archaea

Archaea is a relatively new domain, since these organisms used to be grouped with the bacteria. There are however some similarities which include unicellular nature, cells lack a nucleus or any other organelle, they have 70S ribosomes, and all Archaea are microbes. The structure of their cell wall is completely different from the bacteria cell wall (notably lacking peptidoglycan but having pseudomurein instead). In addition, their rRNA sequences have shown that they are not closely related to Bacteria at all.

Many archaea live in habitats that have very high temperatures, low pH, concentrated salts, or are completely lacking oxygen. However, some archaea are members of the human microbiome, residing in the digestive tract and oral cavity. Thus, they cannot be termed to be exclusively extremophiles.

Eukarya

The Eukarya domain includes many non-microbes, such as animals and plants, as well as microbial examples such as fungi, protists, slime moulds, and bread moulds. The eukaryotic cell type has a nucleus, as well as many organelles, such as mitochondria or an endoplasmic reticulum. They have 80S ribosomes and are commonly found as unicellular or multicellular.

Viruses

Viruses are not part of the three Domain Classification, as they lack ribosomes and therefore lack rRNA sequences for comparison between the members of the three Domains. They are classified separately, using characteristics specific to viruses. Viruses are typically described as “obligate intracellular parasites,” a reference to their strict requirement for a host cell in order to replicate or increase in number. These acellular entities are often agents of disease, as a result of their lifestyle of host cell invasion.

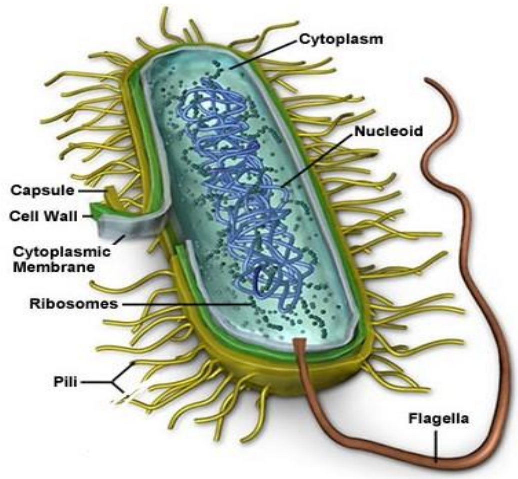
Practice Questions

1. What is the basis for Woese’s classification and what are the three domains?

2. What are the basic characteristics of members of the three domains? Where do microbes fit in?

3. What are the basic characteristics of viruses? Why are they not classified in one of the three domains?

STRUCTURE OF THE BACTERIAL CELLS



A typical bacterial cell

Source: https://micro.magnet.fsu.edu/cells/bacteriacell.html

Bacteria are prokaryotes, lacking well-defined nuclei and membrane-bound organelles. They come in many shapes and sizes, from minute spheres, cylinders and spiral threads, to flagellated rods, and filamentous chains.

Capsule - The capsule is made up of polysaccharides (complex carbohydrates). The most important role it plays is to keep the bacterium from drying out and to protect it from phagocytosis (engulfing) by larger microorganisms. The capsule is a major virulence factor in the major disease-causing bacteria, such as *Escherichia coli* and *Streptococcus pneumoniae*. Non-encapsulated mutants (cells without capsule) of these organisms are avirulent i.e. do not cause disease.

Cell Wall - Each bacterium is enclosed by a rigid cell wall composed of peptidoglycan, a protein-sugar (polysaccharide) molecule. The wall gives the cell its shape and surrounds the cytoplasmic membrane, protecting it from the environment. It also helps to anchor appendages such as **pili** and **flagella**, which originate in the cytoplasm membrane and protrude through the wall to the outside. The strength of the wall is responsible for keeping the cell from bursting when there are large differences in osmotic pressure between the cytoplasm and the environment.

Cell wall composition varies widely amongst bacteria and is one of the most important factors in bacterial species analysis to differentiate between the two forms. When exposed to a gram stain, gram-positive bacteria retain the purple color of the stain because the structure of their cell walls traps the dye. In gram-negative bacteria, the cell wall is thin and releases the dye readily when washed with an alcohol or acetone solution.

Cytoplasmic Membrane – This is a layer of phospholipids and proteins which is also called the plasma membrane or cell membrane encloses the interior of the bacterium, regulating the flow of materials in and out of the cell. This is a structure that bacteria share with all other living cells which acts as a barrier that allows them to selectively interact with their environment. Membranes are highly organized and asymmetric having two sides, each side with a different surface and different functions.

Flagella - Flagella (singular: flagellum) are whip-like structures that provide a means of locomotion for those bacteria that have them. They can be found at either or both ends of a bacterium or all over its surface. The flagella beat in a propeller-like motion to help the bacterium move toward nutrients, away from toxic chemicals or toward the light in the case of the photosynthetic cyanobacteria.

Nucleoid - The nucleoid is a region of cytoplasm where the chromosomal DNA is located. It is not a membrane bound nucleus, but simply an area of the cytoplasm where the strands of DNA are found. Most bacteria have a single, circular chromosome that is responsible for replication, although a few species do have two or more. Smaller circular extracellular DNA strands called plasmids are also found in the cytoplasm of some bacteria.

Pili - Many species of bacteria have pili (singular: pilus) which are small hair-like projections emerging from the outside cell surface. These outgrowths assist the bacteria in attaching to other cells and surfaces, such as teeth, intestines, and rocks. Without pili, many disease-causing bacteria lose their ability to infect because they are unable to attach to host tissue. Specialized pili are used for conjugation during which two bacteria exchange fragments of plasmid DNA.

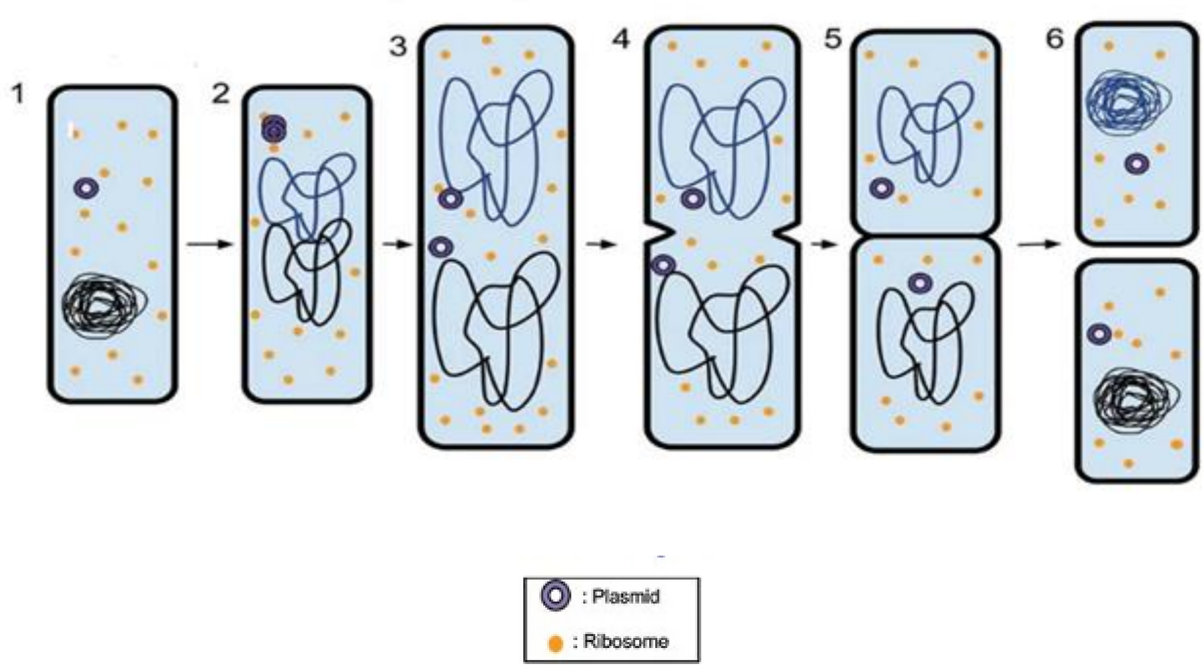
Ribosomes - These are microscopic "factories" found in all cells, including bacteria. They translate the genetic code from the molecular language of nucleic acid to that of amino acids which is the building blocks of proteins. Proteins are the molecules that perform all the functions of cells and living organisms. Bacterial ribosomes are similar to those of eukaryotes, but are smaller and have a slightly different composition and molecular structure. Bacterial ribosomes are never bound to other organelles as they sometimes are (bound to the endoplasmic reticulum) in eukaryotes, but are free-standing structures distributed throughout the cytoplasm.

MICROBIAL REPRODUCTION AND GROWTH

During their growth cycles, microorganisms undergo reproduction many times, causing the numbers in the population to increase faster than in most eukaryotic cells. In fungi, unicellular algae, and protozoa, reproduction involves a duplication of the nucleus through the asexual process of mitosis and a splitting of the cell in cytokinesis. Reproduction can also occur by a sexual process in which haploid nuclei unite to form a diploid cell having two sets of chromosomes. Various changes then follow to yield a sexually produced offspring. Sexual reproduction has the advantage of mixing chromosomes to obtain genetic variations not possible with asexual reproduction. However, fewer individuals normally result from sexual reproduction than from asexual reproduction.

Binary Fission

Bacteria reproduce by the asexual process of **binary fission.** In this process, the chromosomal DNA duplicates, after which the bacterial membrane and cell wall grow inward to meet one another and divide the cell in two. The two cells separate and the process is complete. Binary fission is the mode of reproduction in Archaea and some eukaryotes like Amoeba and *Paramecium*.



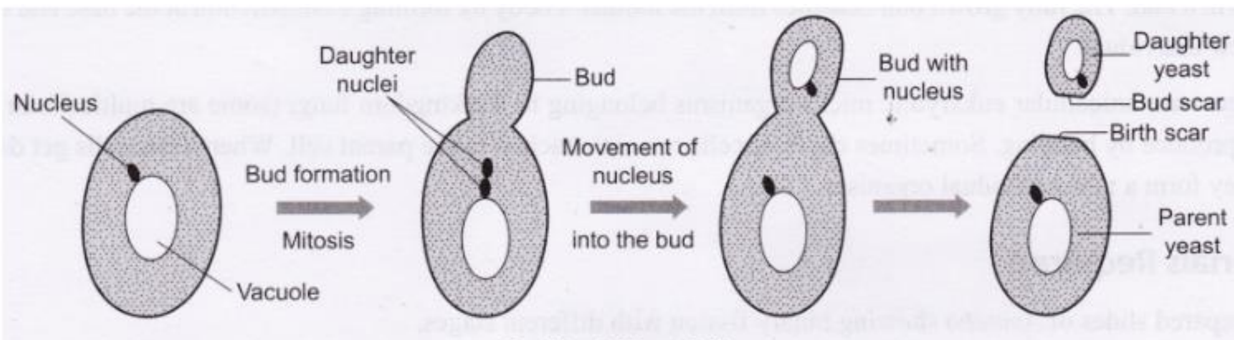
Binary fission in Bacteria

Image source: https://microbenotes.com/binary-fission/

Budding

This is another form of asexual reproduction in which a new organism develops from an outgrowth or bud due to cell division at a particular site. The new organism remains attached as it grows but separate from the parent cell when it is matured leaving behind scar tissue. Since the reproduction is asexual, the newly created organism is a clone and is genetically identical to the parent organism. Unlike binary fission, where the parent cell divides symmetrically into two roughly equal daughter cells, budding involves the formation of a new cell while the parent cell remains largely unchanged.

The process begins with the formation of a small protrusion or bud on the surface of the parent cell. This bud grows gradually by accumulating cellular materials such as cytoplasm and genetic material. As the bud enlarges, the parent cell provides the necessary components for the development of the daughter cell. Once the bud reaches a certain size and maturity, it eventually detaches from the parent cell to become an independent daughter cell.



Budding in Yeast

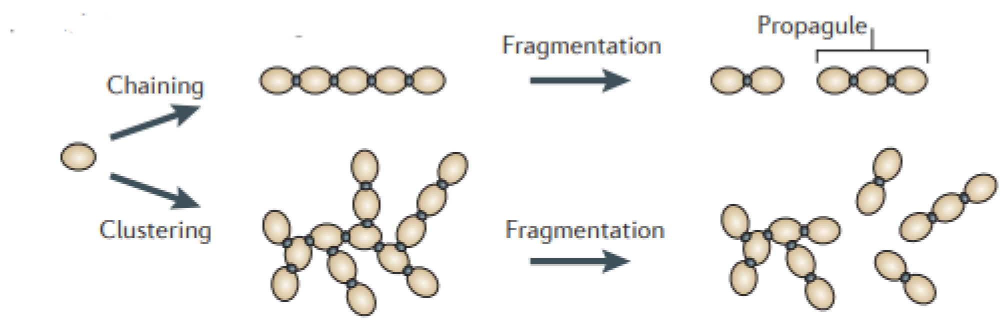
Image source: https://www.cbsetuts.com/ncert-class-10-science-lab-manual-binary-fission-amoeba-budding-yeast/

This mode of reproduction is observed in yeast and bacteria such as *Caulobacter* and *Hyphomicrobium*, and allows them to exploit their environments effectively by generating offspring without the need for significant cellular reorganization or division of the entire cell body

Fragmentation

Some filamentous bacteria and fungi reproduce by this mode of asexual reproduction where a parent organism breaks into fragments, each capable of growing into a new individual. In bacteria, each fragment contains a part of the cytoplasm and genetic material. These fragments can grow and develop into new bacterial cells, each identical to the original parent cell. This type of reproduction is less common than binary fission but can be observed in some filamentous bacteria such as *Nostoc, Anabaena*.

In fungi, fragmentation is a more frequent mode of reproduction. Fungal **mycelium**, the network of **hyphae** that makes up the vegetative part of the fungus, can break into smaller pieces due to mechanical disturbances, environmental conditions, or naturally as part of the organism's lifecycle. Each fragment of the mycelium that contains sufficient resources and suitable conditions can grow into a new mycelium. This form of reproduction ensures the rapid spread and colonization of the fungus in a given environment. Examples include molds and some species of mushrooms.



Fragmentation of cells

Image source: Claessen, D., Rozen, D., Kuipers, O. *et al.* Bacterial solutions to multicellularity: A tale of biofilms, filaments and fruiting bodies. *Nat Rev Microbiol* **12**, 115–124 (2014). https://doi.org/10.1038/nrmicro3178

One of the special characteristics of bacteria is the relatively short **generation time** (the time required for a microbial population to double in numbers). The generation time varies among bacteria and often ranges between 30 minutes and three hours. Certain bacteria have very brief generation times. For example the generation time of *Escherichia coli* is about 20 minutes under optimal conditions.

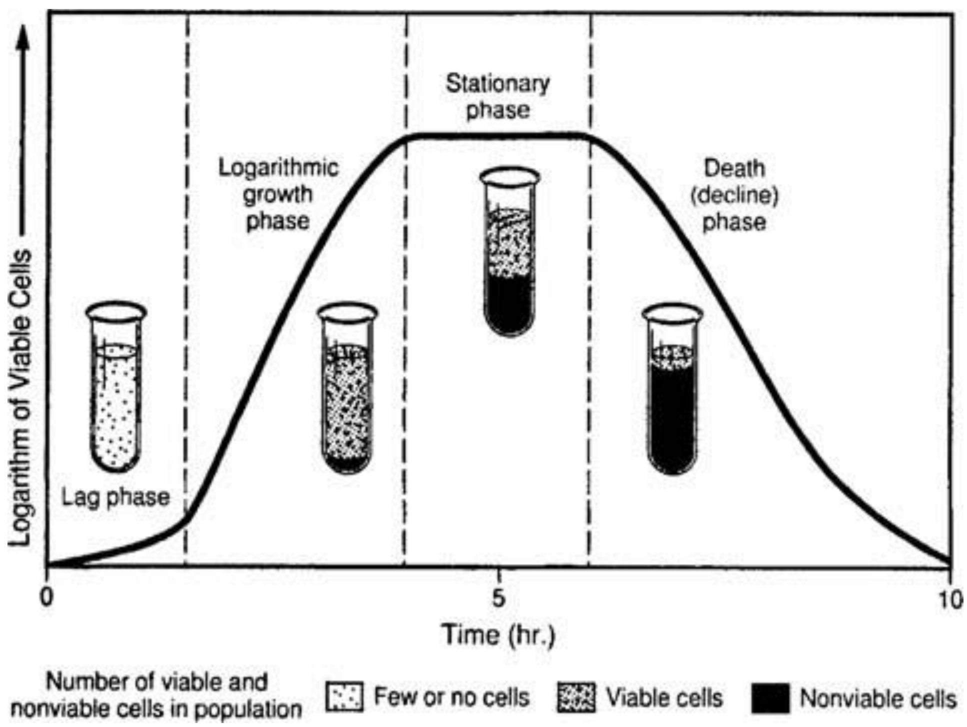
The growth curve

The growth of a bacterial population can be expressed in various phases of a **growth curve.** The logarithms of the actual numbers in the population are plotted in the growth curve along the side axis, and the time is plotted at the base. Four phases of growth are recognized in the growth curve.

In the first phase, called the **lag phase**, the population remains at the same number as the bacteria become accustomed to their new environment. Metabolic activity is taking place, and new cells are being produced to offset those that are dying.

In the **logarithmic phase**, or **log phase**, bacterial growth occurs at its optimal level and the population doubles rapidly. This phase is represented by a straight line, and the population is at its metabolic peak.

During the next phase, the **stationary phase**, the rate of reproduction equals the rate of bacterial cell death, resulting in a stagnation of the population size. During this phase, the bacterial population reaches a plateau, indicating that no net increase in cell numbers occurs. Factors contributing to bacterial death during this phase encompass the buildup of waste products, nutrient depletion, and the emergence of adverse environmental conditions. If the conditions are not altered, the population will enter its **decline**, or **death phase**. In this phase, the bacteria die off rapidly, the curve turns downward.



Growth curve of bacterial population showing the four major phases.

PROKARYOTES AND EUKARYOTES

Prokaryotic Cells

The term “prokaryote” is derived from the Greek word “*pro*“, (meaning: before or first) and “*karyon*” (meaning: kernel or nut). It translates to “before nuclei“.

Prokaryotic cells are comparatively smaller and much simpler than eukaryotic cells. The other defining characteristic of prokaryotic cells is that they do not have membrane-bound cell organelles such as a nucleus. The nucleoid which makes up their genetic material floats freely in the cytoplasm. Prokaryotes are always unicellular and they include bacteria and Archaea. Their mode of reproduction is majorly through the process of binary fission.

Prokaryotes possess a capsule that encloses their entire structure, serving as a protective covering. This feature plays a critical role in preventing phagocytosis, the process by which bacteria are engulfed by other eukaryotic cells, such as macrophages. Additionally, prokaryotes typically possess pilus, located on their external surface, aiding in attachment to diverse environments. These pili, often referred to as attachment pili, resist displacement, contributing to their effectiveness in bacterial attachment. This characteristic is commonly observed across various bacterial species. Directly beneath the protective coating, the cell wall offers structural support and rigidity to the cell. Situated beneath the cell wall is the plasma membrane, which limits or regulates the entry and exit of material into the interior contents of the cell while the cytoplasm facilitates cellular growth. Within the cytoplasm are ribosomes involved in protein synthesis. Ribosomes are among the smallest components within the cell but are integral to its fundamental processes.

Most prokaryotes also contain plasmids, which contain small, circular pieces of DNA. To help with locomotion, flagella are present, although, pilus can also serve as an aid for locomotion. Common examples of Prokaryotic organisms are bacteria and Archaea. Also, all members of Kingdom Monera are prokaryotes.

Eukaryotic Cells

The term “**Eukaryotes**” is derived from the Greek word “*eu*“, (meaning: true or good) and “*karyon*” (meaning: kernel or nut), therefore, translating to “good or true nuclei.” Eukaryotes are more complex and much larger than prokaryotes. They include almost all the major kingdoms except kingdom Monera.

Structurally, eukaryotes possess a cell wall, which supports and protects the plasma membrane. The cell is surrounded by the plasma membrane and it controls the entry and exit of certain substances.

The nucleus encloses DNA which is responsible for storing all genetic information. The nucleus is enveloped by the nuclear membrane. Inside the nucleus, the nucleolus is involved in the assembly of the cell’s ribosome. Eukaryotic cells also contain mitochondria, which are responsible for the creation of energy utilized by the cell. Chloroplasts are the subcellular sites and organelles of photosynthesis and are present in only plant cells. The endoplasmic reticulum aids in material transportation within the cell. Additionally, various other organelles perform diverse functions, including ribosomes, lysosomes, Golgi bodies, cytoplasm, chromosomes, vacuoles, and centrosomes. Examples of eukaryotes include almost every unicellular organism with a nucleus and all multicellular organisms.

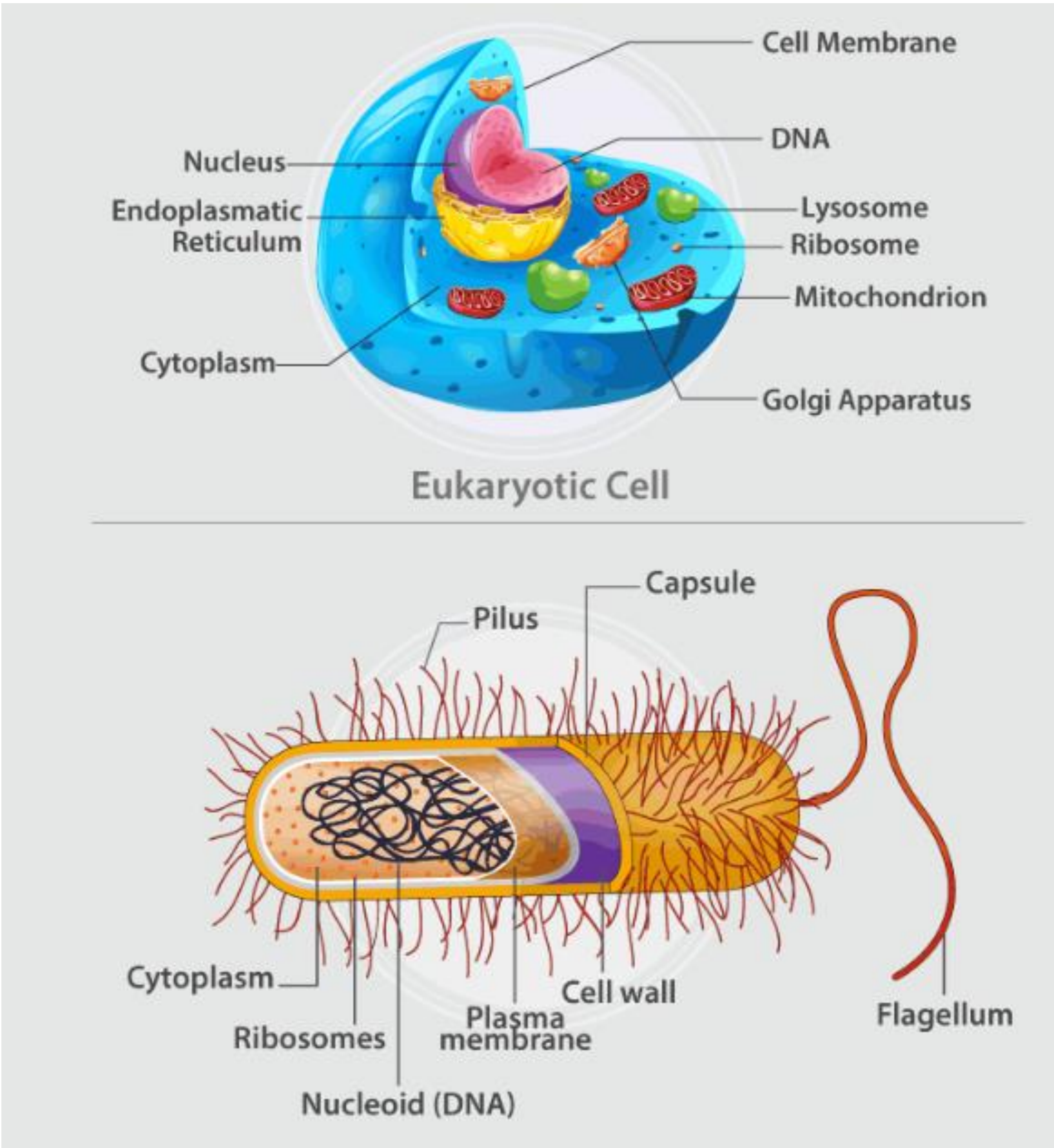


Image illustrating the difference between Prokaryotic and Eukaryotic Cells
Source: https://byjus.com/biology/prokaryotic-and-eukaryotic-cells/

DISTRIBUTION OF MICROORGANISMS IN NATURE

Microorganisms are ubiquitous i.e. they are distributed across various natural environments. The extensive distribution of microorganisms is attributed to their physiological diversity, which allows them to use a wide range of inorganic and organic compounds for sustenance of their life processes. Their distribution and abundance are however influenced by a multitude of factors including physical, chemical, and biological parameters. Microbes are found in every kind of habitat (terrestrial, aquatic, atmospheric, or living host) and their presence in turn affects the environment in which they grow.

Microorganisms in the atmosphere

The atmosphere is made up of a mixture of gases surrounding the biosphere. The atmosphere is the layer nearest to the earth and it contains all major groups of microbes ranging from algae to the viruses. So, in addition to gases, dust particles and water vapour, air also contains microorganisms. There are vegetative cells and spores of bacteria, fungi and algae, viruses and protozoan cysts. Since air is often exposed to sunlight, it has a higher temperature and less moisture. There is a gradual increase in the death rate with an increase in temperature from 18°C to 49°C and also low and high relative humidity cause the death of most microorganisms. So, most of these microbial forms will not survive this conditions.

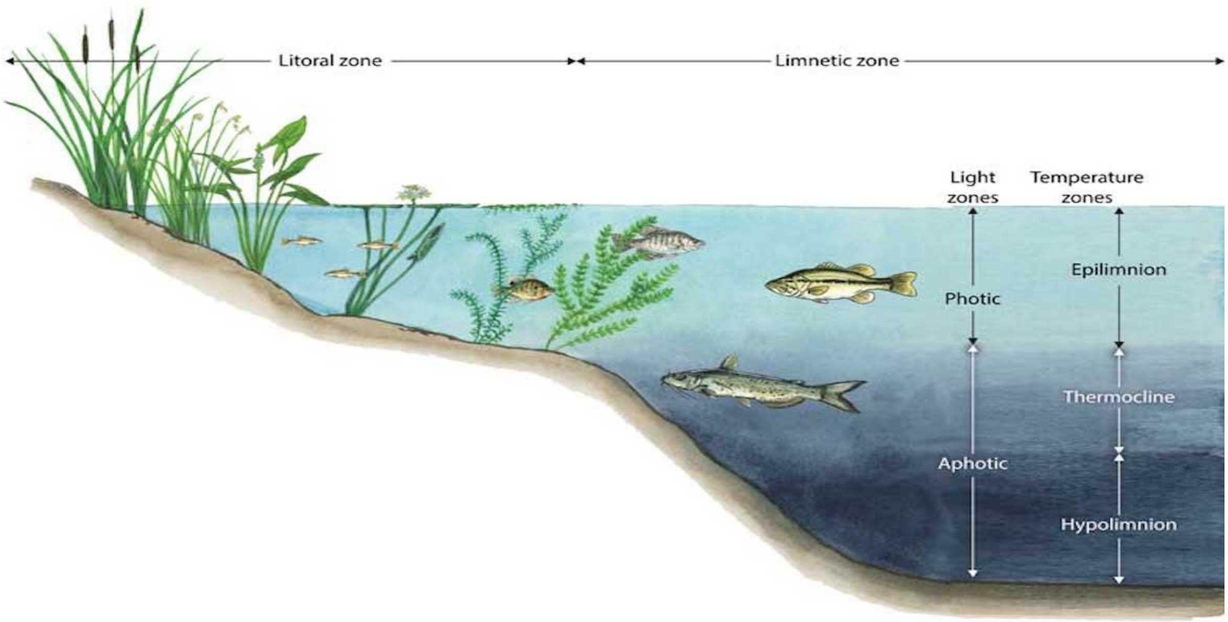
Air current is also important in the dispersal of microorganisms as it carries them over a long distance. In still air, the particles with microorganisms tend to settle down. But a gentle air can keep them in suspension for long periods. Air is not a medium in which organism grow but is a carrier of particulate matter, dust particles, spores. Air is mainly a transport medium for microorganisms as most cells in the atmosphere are metabolically inactive during this phase. Microbes occur in small numbers in air when compared with soil or water. The outdoor and indoor air microflora can be different due to some peculiarities of these environments.

One of the most common sources of outdoor microflora is the soil and water. Microorganisms found in water can become airborne, typically through water droplets released from plant or animal surfaces. Factors such as weather conditions, seasons, and geographical locations influence the composition and concentration of outdoor microflora. Important sources of indoor microflora are human activities such as coughing, sneezing, talking, and laughing. The type of surfaces, cleanliness, humidity, and temperature within an indoor space affects the diversity and abundance of indoor microflora.

Microorganisms in the aquatic environment

Microorganisms are found in all aquatic environments, from freshwater to saltwater, and from the surface to the depths of the ocean. They play a vital role in the functioning of aquatic ecosystems, and their distribution is influenced by numerous environmental factors such as nutrient availability, depth, temperature, salinity, and human activities. The roles microbes play in nutrient cycling, organic matter decomposition, and as primary producers and consumers

shows their importance in the aquatic ecosystems. Microorganisms found in the aquatic environments include bacteria, archaea, viruses, algae, protozoa, and fungi.
The aquatic environment includes inland surface water (lakes, streams, and rivers), seas, and ground water. In freshwater habitats, the population and distribution of microorganisms are influenced by a variety of environmental factors, including temperature, light availability, nutrient concentrations, water flow, and the presence of organic matter. These factors contribute to the diverse and dynamic microbial communities found in lakes, rivers, streams, ponds, and wetlands.



The stratification of a freshwater environment
Source: https://kascomarine.com/blog/pond-lake-zone-identification/

In freshwater lakes, microbial populations exhibit vertical stratification due to variations in light and oxygen levels with depth. The upper layer, or epilimnion, is well-lit and oxygen-rich, supporting a high density of photosynthetic microorganisms such as cyanobacteria and green algae. These primary producers form the base of the food web, converting sunlight into organic matter through photosynthesis. Below the epilimnion is the metalimnion or thermocline, a transition zone where temperature and light decrease rapidly with depth. Microbial activity in this layer includes both photosynthetic and heterotrophic processes. The lower layer, or hypolimnion, is typically dark and may become anoxic (devoid of oxygen) in deeper lakes, supporting anaerobic microorganisms such as sulfate-reducing bacteria and methanogens that thrive in low-oxygen conditions.
Rivers and streams, characterized by flowing water, have microbial populations that are constantly influenced by the surrounding environment and upstream inputs. In these habitats, biofilms (communities of microorganisms attached to surfaces such as rocks and plant roots) are common. These biofilms include bacteria, algae, and fungi, and play crucial roles in nutrient cycling and organic matter decomposition. The composition of microbial communities in rivers and streams varies along the flow gradient, with upstream areas typically having different

microbial assemblages compared to downstream regions due to differences in nutrient availability, flow rate, and substrate types. Also, in freshwater environments, microbial populations are also influenced by seasonal variations. During warmer months, increased temperatures and light availability can lead to algal blooms, where the rapid growth of algae, including cyanobacteria, significantly alters the microbial community structure. On the contrary, in colder months, microbial activity may decrease, and the composition of microbial communities can shift toward species adapted to lower temperatures.

The distribution of microorganisms in freshwater habitats is further influenced by the input of nutrients from surrounding land, which can lead to eutrophication. Eutrophication which is characterized by high nutrient levels, often result in increased microbial biomass and shifts in community composition, favoring fast-growing, nutrient-loving species. In contrast, oligotrophic conditions, with low nutrient levels, support more specialized and slower-growing microorganisms.

Human activities, such as agriculture, urbanization, and industrial processes, also impact microbial populations and distribution in freshwater habitats. Runoff containing pollutants, nutrients, and organic matter can alter microbial community structure and function, leading to shifts in ecosystem processes.

The marine habitat comprising of the oceans, seas, estuaries is characterized by high salt content in comparison with the fresh water habitat. The population and distribution of microorganisms are influenced by a range of environmental factors such as light availability, nutrient concentrations, temperature, salinity, pressure, and the presence of organic matter. These factors create diverse and complex microbial communities across different marine environments such as the open ocean, coastal areas, deep-sea regions, and hydrothermal vents. The total bacterial numbers generally being about ten times higher in coastal waters compared to the open ocean.

The open ocean, also known as the pelagic zone, has microbial populations in vertical strata due to variations in light and nutrients. The euphotic zone, extending from the surface to depths where light can penetrate, supports high densities of photosynthetic microorganisms, primarily phytoplankton, including diatoms, dinoflagellates, and cyanobacteria. These primary producers are crucial for carbon fixation and form the base of the marine food web. Below the euphotic zone, in the aphotic zone, where light does not penetrate, microbial life is dominated by heterotrophic bacteria and archaea that rely on organic matter sinking from the upper layers for energy.

The Coastal areas are characterized by nutrient-rich waters and high primary productivity, support dense and diverse microbial communities. These areas often experience significant inputs of organic and inorganic nutrients from terrestrial sources, which can lead to high microbial biomass and activity.

In the deep sea, microbial populations are influenced by high pressure, low temperatures, and the absence of light. Despite these harsh conditions, the deep ocean hosts a variety of microorganisms, including barophiles (pressure-loving bacteria and archaea) and psychrophiles (cold-loving microorganisms). These microbes play vital roles in the decomposition of organic matter and nutrient cycling in deep-sea ecosystems. The deep-sea floor, or benthic zone, also harbors diverse microbial communities, particularly around sediment-rich areas where organic matter accumulates.

Hydrothermal vents are unique marine habitats that support specialized microbial communities adapted to extreme conditions. Hydrothermal vents, found along mid-ocean ridges, emit mineral-rich, hot water that supports chemosynthetic bacteria and archaea. These microorganisms derive energy from the oxidation of inorganic compounds such as hydrogen sulfide, methane, and reduced metals, forming the basis of a unique food web that includes vent-specific fauna. Cold seeps, which release methane and hydrogen sulfide at the seafloor, also host chemosynthetic microbial communities that play key roles in methane oxidation and sulfur cycling.

In the groundwater environment, microorganisms are the sole inhabitants and bacteria are the dominant type of microbes present, however, the levels of microbial activity are low. This is because as the ground water collects in permeable rocks below the water table, many of the nutrients are filtered out. Because of this, ground water is only able to support a limited population of micro-organisms.

Microorganisms in the terrestrial environment

Microorganisms in terrestrial environments exhibit varied populations and distributions, significantly influenced by factors such as soil type, climate, vegetation, organic matter availability, and human activities. These microorganisms are integral to ecosystem functions including nutrient cycling, organic matter decomposition, soil structure maintenance, and plant growth promotion. Their primary habitats within terrestrial environments include soil, plant roots, plant surfaces, and decaying organic matter.

In soil, the distribution of microorganisms is highly diverse. Soil provides a complex and dynamic habitat for a different populations of microorganisms, including bacteria, archaea, fungi, algae, protozoa, and viruses. Bacteria are the most abundant microorganisms in soil, with populations reaching up to billions of cells per gram of soil. Archaea, while less abundant than bacteria, play significant roles in nutrient cycling, particularly in extreme environments such as highly saline soils or hot springs. Key groups of soil archaea include methanogens and nitrifying archaea.

Fungi are also critical components of soil microbial communities. They are the primary decomposers of complex organic materials like lignin and cellulose, contributing to the formation of humus and soil structure. Fungal populations are typically lower in number than bacterial populations but can dominate soil biomass due to their larger cell size.

Algae and cyanobacteria are present in soil, especially in surface layers where light is available. These photosynthetic microorganisms contribute to primary production and the formation of soil crusts in dry regions. Protozoa, such as amoebae, ciliates, and flagellates, are crucial in regulating bacterial populations and nutrient cycling through their predation activities.

Viruses, particularly bacteriophages, are abundant in soil and play significant roles in controlling bacterial populations.

STERILIZATION AND DISINFECTION

Sterilization is the complete elimination of all forms of microbial life both vegetative and spore forms from a surface or an object. After sterilization, an object is referred to as being sterile or aseptic. Sterilization is carried out by various physical and chemical methods.

Classification of Sterilization

Sterilization is achieved by different physical and chemical methods in microbiology.

Physical methods of sterilization

This class of sterilization method includes the following:

Heat Sterilization

Heat sterilization is one of the most effective methods of achieving the complete the destruction of cell constituents and enzymes. It is done by two methods.

Moist Heat Sterilization: Moist heat sterilization is done with the help of an instrument called an autoclave. An autoclave works on the principle of producing steam under pressure. Thus moist heat sterilization is also known as steam sterilization. The water is boiled in an autoclave at 121-134℃ at a pressure of 15psi for 15 minutes. The high pressure increases the boiling point of water and helps to achieve a higher temperature for sterilization. The high pressure also help to increase the rapid penetration of heat into deeper parts of the material while the moisture leads to the coagulation of proteins in the microorganism, and they are effectively removed. Moist heat is suitable for heat-stable materials that can withstand high temperatures, pressure and moisture such as glassware, culture media, dressings, surgical and diagnostic equipment and aqueous injections

Dry Heat Sterilization: This method is used on objects that are sensitive to moisture. Moisture-free heat or dry heat is applied on the surface or objects such that there is denaturation protein which leads to oxidative damage, and ultimately the microbial cell dies out. Some methods of dry heat sterilization include incinerators, hot air ovens and flaming techniques.

Filtration

This method uses membranous filters with small pores between 0.2-0.45 µm to filter out liquid so that all the bigger particles and microbes cannot pass through. Filtration does not kill microbes, it separates them out. It is used to remove microbes from heat labile liquids solutions that cannot be autoclaved such as serum, antibiotic solutions, sugar solutions, urea solution. Various applications of filtration include removing bacteria from ingredients of culture media, preparing suspensions of viruses and phages free of bacteria, measuring sizes of viruses, separating toxins from culture filtrates, counting bacteria, and clarifying fluids. Filtration is aided by using either positive or negative pressure using vacuum pumps.

Irradiation
Irradiation is the process of exposing surfaces or objects to different kinds of radiation for sterilization. The major target of the radiation is usually the microbial DNA which undergoes mutation upon exposure to such electromagnetic radiation. It is of two types:
Non-ionising Radiation: Ultraviolet radiation is exposed to the object, which is absorbed by nucleic acids of the microorganisms. This leads to error during DNA replication and eventually, cell death.
Ionising Radiation: Upon exposure to ionising radiations such as gamma rays and X-rays, reactive oxygen species such as hydrogen peroxide and superoxide ions are formed that oxidise the cellular components of the microbe, and they die.

Ultrasonic wave
Sound waves of frequency >20,000 cycle/second kills bacteria and some viruses on exposure for an hour. Microwaves are not particularly antimicrobial in themselves, rather the killing effect of microwaves are largely due to the heat that they generate. High frequency sound waves disrupt cells. They are used to clean and disinfect instruments as well as to reduce microbial load. This method is not reliable since many viruses are not affected by these waves.

Chemical methods of sterilization

This method involves the use chemical to sterilize surfaces or objects sensitive to high temperature, moisture and those prone to damage by irradiation especially materials made of plastics or rubber and some biological specimens. This method uses chemical agents in liquid or gaseous form to achieve sterility.
Gaseous sterilization involves the process of exposing equipment or devices to sterilizing gases in a closed heated or pressurized chamber. It is a more effective technique as gases can pass through a tiny orifice and provide more effective results. The gases are commonly used along with heat treatment to facilitate the functioning of the gases. However, there is an issue of release of some toxic gases during the process which needs to be removed regularly from the system. The gaseous chemical agents used for sterilization include ethylene oxide, formaldehyde, nitrogen dioxide and ozone.
Liquid sterilization involves the submerging of equipment in the liquid sterilant to eliminate all viable microorganisms and their spores. Although liquid sterilization is not as effective as gaseous sterilization, it is appropriate in conditions where a low level of contamination is present. Common liquid chemical agents that are used for sterilization include hydrogen peroxide, glutaraldehyde and hypochlorite solution.

Disinfection
Disinfection is defined as the complete elimination of microorganisms, except bacterial spores, on inanimate objects. Unlike sterilization, which aims to kill all forms of microbial life, disinfection does not necessarily eliminate all microorganisms, particularly resistant bacterial spores. The primary goal of disinfection is to reduce the risk of infection by pathogens. The types of disinfectants include

Alcohols: Ethanol and isopropanol are commonly used for their effectiveness against a wide range of microorganisms. They are particularly effective against vegetative bacteria, fungi, and some viruses. Alcohols are used for disinfecting surfaces, equipment, and skin. However, they are not effective against bacterial spores and have limited residual activity.

Aldehydes: Glutaraldehyde and formaldehyde are high-level disinfectants used for medical equipment and instruments. They are effective against bacteria, viruses, fungi, and spores. However, their use requires careful handling due to their toxic and irritating properties.

Oxidizing Agents: Hydrogen peroxide and peracetic acid are strong oxidizing agents effective against a broad spectrum of microorganisms, including spores. They are used for surface disinfection, equipment sterilization, and in some cases, environmental disinfection.

Halogens: Chlorine compounds (e.g., sodium hypochlorite) and iodine compounds (e.g., iodophors) are widely used disinfectants. Chlorine is effective against bacteria, viruses, and fungi, and is commonly used for water disinfection. Iodophors are used for skin disinfection and surface cleaning.

Quaternary Ammonium Compounds (Quats): These compounds are effective against bacteria, fungi, and enveloped viruses. They are used for surface disinfection in healthcare settings and food processing environments. However, they are less effective against non-enveloped viruses and spores.

Phenolics: Phenol and its derivatives are used for disinfecting surfaces and equipment. They are effective against bacteria and fungi but have limited activity against viruses and spores.