

## Chapter I: The Microbial World

### I. Introduction

- **Micro-** prefix: from the Greek "*mikros*", meaning "small."
- **Bio-** prefix: from the Greek "*bios*", meaning "life."
- **Logy:** from the Greek "*logia*", meaning "theory, science."

Microbiology is a subdiscipline of biology that studies microorganisms.

The term microorganism or microbe does not have a precise taxonomic meaning. It refers to tiny organisms that are invisible to the naked eye but visible under an optical microscope or an electron microscope. These include bacteria, archaea, and eukaryotes, distributed across the three domains of life. These organisms are highly diverse in terms of shape, size, and lifestyle. Their small size requires the use of special techniques, such as microscopy, to observe them.

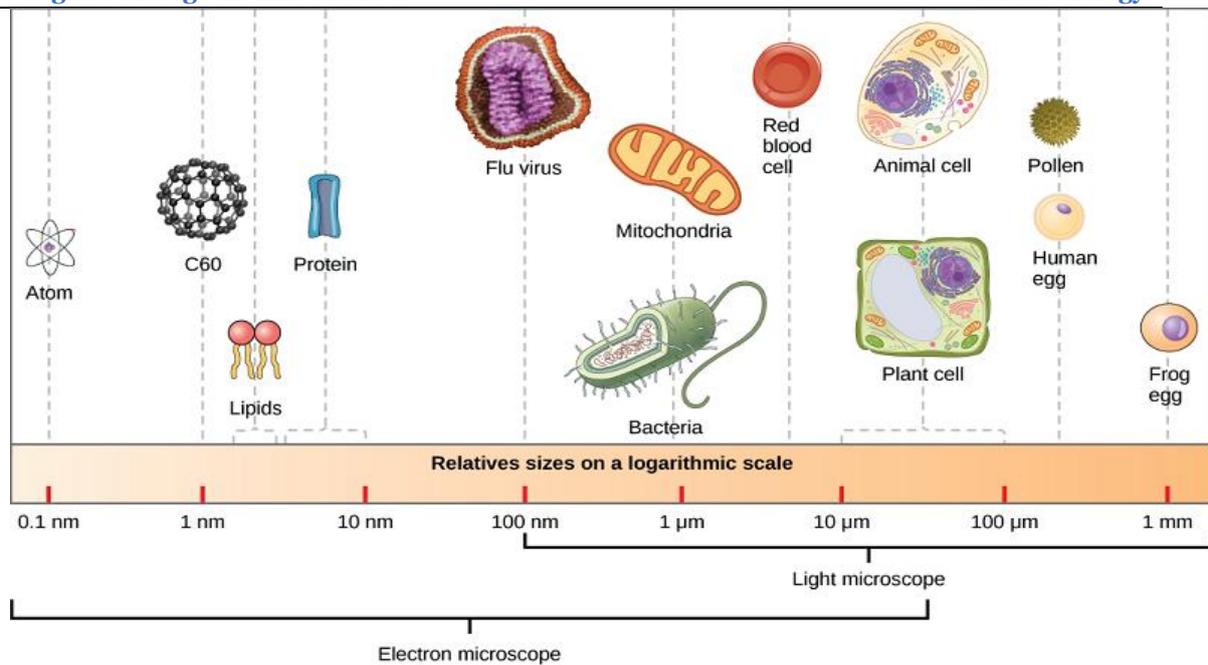
Microbiology is divided into several branches based on the type of microbe studied:

- **Bacteriology:** focuses primarily on bacteria but is often used synonymously with microbiology.
- **Protozoology:** deals with protozoa and is a branch of parasitology that studies protozoan parasites of humans.
- **Mycology:** studies fungi.
- **Phycology or Algology:** studies algae.
- **Virology:** studies viruses.

Microbiology encompasses the study of various aspects of microorganisms, including their structure, function, classification, ecology, reproduction, genetics, and interactions with their environment and other living organisms, including humans.

Microbiology plays an essential role in numerous areas of science and society. It contributes to :

- The prevention and treatment of infectious diseases.
- The production of safe food.
- The development of new medical therapies.
- The discovery of new antibiotics.
- The detection and prevention of epidemics.
- Environmental protection.
- The sustainable exploitation of natural resources.



## II. History

The history of microbiology dates back to antiquity. Although microorganisms are invisible to the naked eye, early observations and theories about these invisible organisms can be traced to ancient Greece, where thinkers like Hippocrates and Aristotle speculated on the existence of "small creatures" responsible for diseases.

- **1665: Antony Van Leeuwenhoek** (1632–1723), a Dutch cloth merchant, constructed the first microscope by stacking lenses to examine textiles. With his curious mind, he used this instrument to observe particles from his skin, mouth, and teeth, sketching what he saw. He discovered the microbial world. The term "microbe" did not yet exist, and the terminology of the time referred to these observations as "animalcules," including protozoa, unicellular algae, yeasts, etc.
- **1729–1799: Lazzaro Spallanzani** found that boiling broth would sterilize it and kill any microorganisms in it. He also found that new microorganisms could settle only in a broth if the broth was exposed to the air.
- **1857–1876: Louis Pasteur** expanded upon Spallanzani's findings by exposing boiled broths to the air in vessels that contained a filter to prevent all particles from passing through to the growth medium. He also did this in vessels with no filter at all, with air being admitted via a curved tube that prevented dust particles from coming in contact with the broth. By boiling the broth beforehand, Pasteur ensured that no microorganisms survived within the broths at the beginning of his experiment. Nothing grew in the broths in the course of Pasteur's experiment. This meant that the living organisms that grew in such broths came from outside, as spores on dust, rather than spontaneously generated within the broth.

Louis Pasteur demonstrated the roles of microorganisms in lactic and alcoholic fermentation. He developed pasteurization and sterilization techniques, enabling the creation of pure microbial cultures.

These advancements helped disprove the theory of spontaneous generation and supported germ theory instead.

- **1877–1895:** Louis Pasteur proved that certain diseases are caused by microorganisms. This period saw the first systematic research into the origins of certain diseases and the development of vaccines (vaccination was already known since Edward Jenner’s work on smallpox, a viral disease).
- **1873–1882: Robert Koch** discovered that the anthrax bacillus forms resistant endospores capable of remaining in the soil. These endospores were the cause of unexplained “spontaneous” outbreaks of anthrax. Koch revolutionized microbiology by developing techniques for isolating pure cultures, notably the streak plate method, essential for isolating and studying microbes. He also developed techniques to link specific microbes to specific diseases “**Koch’s postulates**” for which he was awarded a Nobel Prize in 1905. These postulates stated that an organism is responsible for a disease if it is present in all sick individuals and absent in healthy ones. These postulates enabled the identification of agents responsible for diseases such as diphtheria, plague, and syphilis. Robert Koch identified the bacillus responsible for tuberculosis (*Mycobacterium tuberculosis*), later known as Koch’s bacillus. Koch also discovered tuberculin, an extract of the bacillus, which played a role in diagnosing the disease.
- **1884:** Hans Christian Gram developed a staining technique (Gram stain), still widely used to classify bacteria into two major groups: Gram-positive and Gram-negative bacteria.
- **1929:** Alexander Fleming discovered the antibacterial properties of penicillin, produced by *Penicillium*. This marked the beginning of the antibiotic era, revolutionizing the treatment of bacterial infections.
- **1944:** Albert Schatz and Selman Waksman discovered another antibiotic, streptomycin, which was soon used to treat tuberculosis.
- **1977:** Carl Woese studied ribosomal RNA and discovered a third form of life, Archaea, genetically distinct from bacteria and eukaryotes.
- **1986:** Using an enzyme from the bacterium *Thermus aquaticus*, Kary Mullis invented the Polymerase Chain Reaction (PCR) technology. PCR became a fundamental tool in molecular biology.
- **1995:** Craig Venter and his colleagues at TIGR sequenced the first complete bacterial genome (*Haemophilus influenzae*), ushering microbiology into the era of genomics.

### III. The Place of Microorganisms in the Living World

With over 500,000 species, the microbial world is extraordinarily diverse. Even today, it is impossible to provide a single, definitive classification of microorganisms. A review of the major microbial groups through their key anatomical and physiological characteristics helps navigate the successive classifications of microorganisms.

Before the discovery of microorganisms, all living beings were classified into the animal and plant kingdoms:

- **Animal organisms** use organic matter as an energy source, store reserves in the form of fats or glycogen, are mobile, and lack cell walls.
- **Plant organisms**, on the other hand, are photosynthetic, using light as an energy source, synthesize starch as a nutritional reserve, are immobile, and have cell walls.

The discovery of new microscopic life forms made it increasingly difficult to classify them within the animal or plant kingdoms. Among these :

- Algae and fungi were more closely related to plants.
- Protozoa, being mobile and non-photosynthetic, were considered animals.
- The placement of bacteria remained unresolved.

To avoid arbitrarily placing unicellular organisms into either kingdom, the German zoologist Haeckel proposed in 1866 a third kingdom, the **Protists** (“*Protista*”), derived from the Greek “*protistos*”, a superlative of *protos*, meaning “**first**.” This kingdom grouped algae, protozoa, and bacteria. In his 1886 version, fungi were also included. Protists are primarily characterized by a rudimentary biological organization.

In 1937, due to the invention of the electron microscope, Edward Chatton distinguished two types of cells:

- **Eukaryotic cells**: with a nucleus surrounded by a membrane and containing cellular organelles.
- **Prokaryotic cells**: with a nucleus lacking a membrane and a very simple organization.

This led to the subdivision of protists into two groups:

- **Lower protists** (“*Protista inferior*”), encompassing prokaryotes (bacteria).
- **Higher protists** (“*Protista superior*”), comprising eukaryotic organisms such as protozoa, fungi, and unicellular algae.

In 1938, H.F. Herbert F. Copeland created a classification system with four kingdoms (based on Haeckel’s classification system): *Monera*, *Protista*, *Plantae*, and *Animalia*. He notably created a new kingdom, *Monera*, which includes bacteria and cyanobacteria, which had previously been classified under the kingdom **Protista**.

The development of molecular biology techniques allowed the characterization of genes encoding ribosomal RNA (rRNA). By comparing numerous 16S rRNA sequences from various living organisms, researchers divided all living organisms into **three domains** and **six kingdoms**:

- ✚ The **Eucarya domain** includes eukaryotic organisms, which can be unicellular or multicellular. It encompasses the following kingdoms :

1. **Kingdom Animalia (Animals)**
2. **Kingdom Plantae (Plants)**

### 3. Kingdom *Fungi* (Mycophyta)

They are non-motile eukaryotes with a classic cell nucleus and rigid chitin-impregnated walls. They contain no photosynthetic pigments and are carbon heterotrophic, that is, they utilize various organic nutrient substrates (in contrast to carbon autotrophic plants). Of the more than 50,000 described fungal species, only about 300 are known to be human pathogens. Fungi reproduce through both asexual (e.g., spore formation, budding) and sexual processes.

Two main morphological categories are distinguished:

- ✓ **Molds (filamentous fungi)**
- ✓ **Yeasts (unicellular fungi)**

### 4. Kingdom *Protista* (Unicellular eukaryotes)

This is a highly diverse kingdom containing all eukaryotes that are not animals, plants, or fungi. It is a paraphyletic group (not a single evolutionary lineage), but for practical purposes, it is useful to classify them based on their similarities to the other three groups.

#### a) Plant-like Protists (Microalgae)

These are autotrophic eukaryotic microorganisms capable of photosynthesis. They possess chloroplasts and, like plants, often have a rigid cell wall composed of cellulose and pectin.

They can be unicellular (e.g., *Chlorella*) or form colonies, filaments, or sheets. Most reproduce asexually through binary fission. Their size typically ranges from 5 to 10  $\mu\text{m}$ .

**Note:** The term "blue-green algae" refers to *Cyanobacteria*, which are prokaryotes belonging to the *Bacteria* domain. Despite their ability to perform photosynthesis, they are not algae and are not part of the *Eucarya* domain.

#### b) Animal-like Protists (Protozoa)

These are heterotrophic unicellular eukaryotes. Their cells resemble animal cells: they lack a cell wall and are typically motile (using flagella, cilia, or pseudopods). They feed by ingesting organic matter (phagocytosis) or absorbing nutrients from their environment. They range in size from 1  $\mu\text{m}$  to several hundred  $\mu\text{m}$ .

Many are free-living in aquatic environments, while others are important parasites of humans and animals.

They most commonly reproduce asexually by binary fission, though sexual reproduction (conjugation, syngamy) can occur in some groups.

#### c) Fungi-like Protists

These protists share several characteristics with fungi. They are heterotrophic and frequently produce spore-bearing structures. However, they are not true fungi, as they differ in important aspects of their cell wall composition and life cycles.

Examples: Myxomycetes (Slime molds) and Oomycetes (Water molds).

**Prokaryotic** living beings are divided into two very different domains:

✚ The **Bacteria domain**, including unicellular organisms known as bacteria. They are unicellular microbes with a simple structure, lacking a nucleus or membrane-bound organelles. They possess a cell wall made of peptidoglycan (found only in bacteria) and have 70S ribosomes. These organisms are ubiquitous, present in soil, water, food, and the human body.

✚ The **Archaea domain**, (from the Greek *arkhaios*, meaning 'ancient'), constitutes a relatively new division in the tree of life. Initially, these organisms were grouped with bacteria and called "*archaebacteria*," a term introduced by Carl Woese in 1977 based on the hypothesis that they were ancient life forms. While this notion of primitiveness has been refined, Woese's groundbreaking comparison of ribosomal RNA (rRNA) sequences revealed that *Archaea* are phylogenetically distinct from both *Bacteria* and *Eukarya*, forming a separate domain of their own.

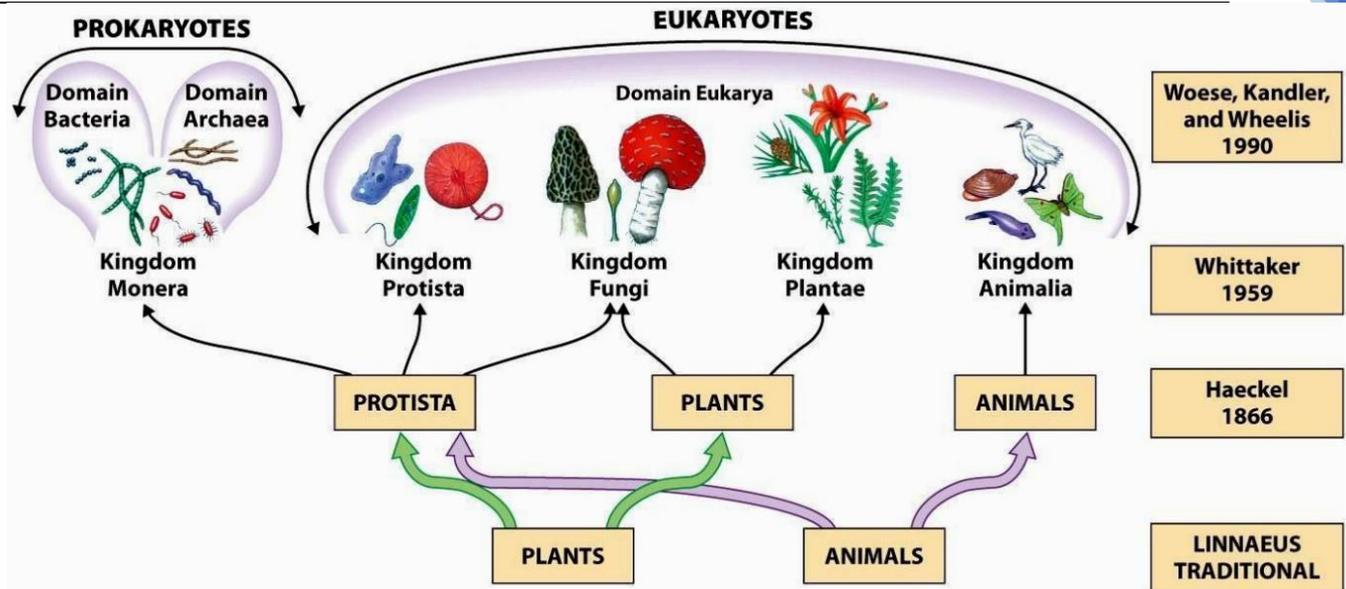
Like *Bacteria*, *Archaea* are mostly unicellular prokaryotes (lacking a true nucleus and membrane-bound organelles), possess 70S ribosomes, and are microscopic. However, they are fundamentally different in several key aspects:

- **Cell Wall:** They completely lack peptidoglycan.
- **Genetics:** Their rRNA sequences are distinctly different, confirming their separate evolutionary path.

*Archaea* exhibit a remarkable diversity of metabolism and are often, but not exclusively, associated with extreme environments. They can be categorized into:

*Archaea* can be categorized into:

- **Methanogens** (e.g., *Methanobrevibacter smithii*), which have specific metabolic pathways, such as methanogenesis.
- **Halophiles** (e.g., *Halobacterium*, *Haloarcula*, *Haloquadrata*), which thrive in environments saturated with salt, such as salt lakes or salt marshes.
- **Thermoacidophiles** (e.g., *Sulfolobus solfataricus*), which live in hot, acidic terrestrial springs.



Classification of the microbial world

## Viruses

All the microorganisms discussed previously share two fundamental characteristics:

- They exhibit a cellular organization.
- They are capable of carrying out their own metabolic activities.

These criteria do not apply to viruses. Unlike other microorganisms, viruses have a non-cellular organization and are unable to generate the energy required to perform vital functions.

Viruses are not included in the Three-Domain Classification system because they lack ribosomes and, consequently, rRNA sequences used for comparison. Instead, they are classified separately. Structurally, viruses consist of nucleic acid (DNA or RNA), which may be single or divided into multiple segments, surrounded by a protein shell called a capsid. In this sense, they can be viewed as entities at the boundary between living organisms and inanimate matter.

Viruses are commonly described as obligate intracellular parasites because they must infect a host cell in order to replicate. These acellular agents often cause disease as a result of invading and multiplying within host cells.

## V. General characteristics of prokaryotic cells

Prokaryotes (*Prokaryota* or *Prokarya*), from the Greek *pro* (*before*) and *karyon* (*nucleus*), exhibit the following characteristics, as identified through advances in electron microscopy, which distinguish them from eukaryotic cells:

### 1. Absence of a nucleus:

Unlike eukaryotic cells, prokaryotic cells lack a nucleus enclosed by a membrane. Their DNA, in the form of a single, naked chromosome, floats freely in the cytoplasm.

## 2. Reduced size:

Prokaryotic cells are generally smaller than eukaryotic cells.

## 3. Limited organelles:

Intracellular organelles such as mitochondria, the endoplasmic reticulum, and the Golgi apparatus are absent in prokaryotic cells. However, they may contain less complex structures, such as ribosomes, plasmids (small circular DNA molecules), and vacuoles.

## 4. Cell wall:

Most prokaryotic cells have a rigid cell wall composed of peptidoglycan (in bacteria). This wall provides mechanical strength and protection.

## 5. Flagella:

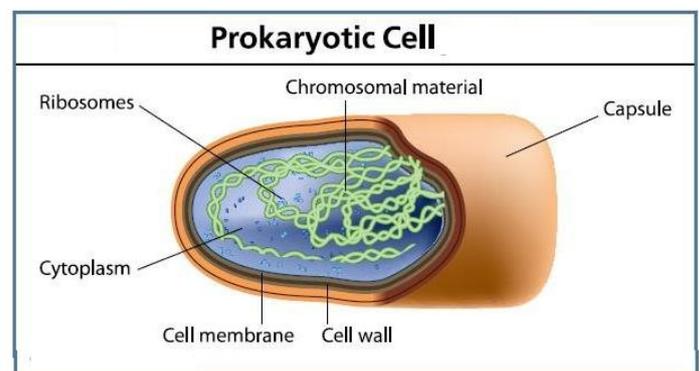
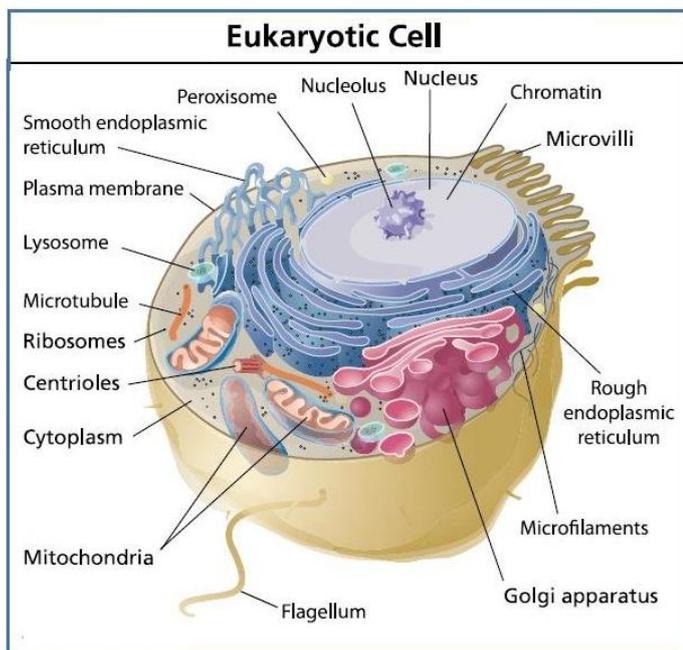
Some prokaryotic cells are equipped with flagella, filamentous structures used for locomotion.

## 6. Asexual reproduction:

Prokaryotic cells primarily reproduce through asexual cell division, specifically binary fission.

## 7. Metabolic diversity:

Prokaryotic cells exhibit significant metabolic diversity. Some are **autotrophic**, capable of producing their own food from inorganic materials, while others are **heterotrophic**, relying on organic sources for nutrition.



Difference between Eukaryotic and Prokaryotic cells