People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research
The Teacher Training College El-Katiba Assia Djebar of Constantine (ENSC)
Natural Sciences Department

General Microbiology:

A Pedagogical Course for Second-Year Natural Sciences Students

By

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Academic Year: 2024-2025

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Introduction

Introduction

بسم الله الرحمن الرحيم والصلاة والسلام على خاتم الأنبياء والمرسلين، وبعد:

Microbiology is the scientific discipline dedicated to the study of microorganisms, defined as living entities too small to be seen with the naked eye. These include bacteria, fungi, protozoa, algae, and viruses. As one of the fundamental pillars of biological sciences, microbiology explores the diversity, structure, function, metabolism, and ecology of these microscopic organisms that exist in virtually every habitat on Earth.

The field of microbiology comprises several specialized branches, including medical microbiology (focusing on pathogenic microorganisms and infectious diseases), environmental microbiology (studying microbes in natural ecosystems), industrial microbiology (utilizing microorganisms for manufacturing processes), and more recently, molecular microbiology (examining the genetic and molecular basis of microbial functions). The discipline employs diverse techniques ranging from traditional culture methods to cutting-edge genomic and proteomic approaches.

The understanding of microorganisms is of crucial importance, not only because of the diseases they cause but also because of their essential roles in maintaining ecological balance, nutrient cycling, and food production. Furthermore, they have become invaluable tools in biotechnological applications. As we continue to face global challenges like antimicrobial resistance, emerging infectious diseases, and environmental degradation, the knowledge gained through microbiological research becomes increasingly vital for developing effective solutions and advancing human welfare.

The present pedagogical work has been specifically prepared for students pursuing General Microbiology in the Natural Sciences Department at the Teacher Higher College El-Katiba Assia Djebar of Constantine (ENSC), who are training to become Natural Sciences educators for middle and secondary school levels. The work has been meticulously designed to serve as a comprehensive resource that presents essential microbiological concepts in a scientifically rigorous yet accessible manner, benefiting all students while fostering a microbiological understanding relevant to various societal contexts.

Complemented by an introduction and a historical view of microbiology, this pedagogical work is divided into eight main Chapters. The Introduction and Historical View part provides a comprehensive overview of microbiology (definition, fields, and applications) and its historical development, highlighting milestones such as Antonie van Leeuwenhoek's discovery of microorganisms and major contributions by pioneers like Pasteur and Koch. Chapter I on the microbial world traces the evolution of microbial taxonomy, explores prokaryotic and eukaryotic

cellular structures, as well as the various microbial groups (bacteria, microscopic fungi, microalgae, and protozoa). Chapter II the morphology and structure of bacteria, covering their diverse shapes, arrangements, and cellular components. It details the external structure of bacterial cells (e.g., cell wall, capsules, flagella, and pili), as well as the internal structures (e.g., nucleoid, ribosomes, and plasmids). Chapter III provides a comprehensive overview of bacterial nutrition and metabolism, emphasizing the nutritional and energy requirements for bacterial growth, the metabolic pathways for carbohydrate, lipid, and protein catabolism, as well as bacterial anabolism with a focus on peptidoglycan synthesis. Chapter IV explores bacterial growth, covering its definition, influence by environmental factors, growth phases in batch cultures, continuous culture systems, as well as methods for estimating bacterial growth. Chapter V provides a comprehensive overview of antimicrobial agents, covering their fundamental principles, classifications, mechanisms of action, and applications. It details physical methods (e.g., heat and radiation), chemical agents (disinfectants and antiseptics), and antibiotics. Chapter VI covers the bacterial taxonomy, highlighting its principles, methodologies, and applications, and discusses the hierarchical classification system, criteria for identifying bacteria, nomenclature rules, as well as the major bacterial groups as defined by Bergey's Manual. Chapter VII explores the roles of microorganisms in industry, health, and the environment and their industrial applications, covering their industrial applications (e.g., metabolites and enzymes production, fermentation and bioreactor operation), emphasizing microbial pathogenicity mechanisms and disease transmissions, and highlighting microbial contributions to biogeochemical cycles and applications. Finally, Chapter VIII on virology covers fundamental virology concepts (historical developments, viral structure, replication cycles, and classification systems) and explores three major virus categories, including bacteriophages, plant viruses, and animal viruses, focusing on their structure, replication, classification, transmission methods, etc.

All information included in this work has been documented through scientific references utilized in the preparation for this essential academic source. It is our sincere hope that this pedagogical work will fulfill its intended educational objectives, "Inshallah", and contribute valuable scientific material to the pedagogical literature in English. The work aims to address the growing demand for accessible scientific references and to serve as a beneficial resource for the broader academic community. May Allah grant success and guidance.

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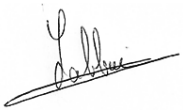


Introduction and Historical View

Introduction and Historical View

1. Introduction to Microbiology

Microbiology is a branch of life sciences that specializes in the study of microorganisms that cannot be seen with the naked eye. These microorganisms are so small that they cannot be observed or examined, and their different shapes cannot be recognized except through a microscope. Commonly referred to as microbes or microscopic organisms, they are considered living entities because they perform all vital functions, such as respiration and reproduction.

The term "Microbiology" derives from three Greek words: "*micros*" (meaning tiny), "*bios*" (meaning life), and "*logos*" (meaning science or study).

1.1. Fields of Microbiology

Microbiology involves the study of a diverse group of microorganisms, including bacteria, microscopic fungi, microalgae, and viruses, through two main perspectives:

- The first approach involves studying microorganisms based on their types, shapes, composition, and functions to define their different characteristics. Its branches are as follows:

- **Bacteriology:** the study of bacteria.
- **Mycology:** the study of fungi.
- **Phycology:** the study of algae.
- **Protozoology:** the study of protozoa.
- **Virology:** the study of viruses.

- The second approach focuses on microbial activity in natural environments and their role in ecosystem functioning, as well as their practical applications. This includes investigating the practical applications of microorganisms (Applied microbiology), such as:

- **Soil Microbiology**

This field examines microorganisms present in soil to understand their vital functions and activities, including their roles in soil fertility, plant preservation, and the recycling of organic materials into inorganic elements. For example, nitrogen fixation (the process of converting atmospheric nitrogen into a form that plants can use) and the conversion of nitrite to nitrate are two key areas of study within this field.

- **Water microbiology**

This area studies microorganisms in various water sources, such as marine and freshwater environments. It examines how these microorganisms affect water quality and ecological balance, investigates sources of contamination and pathogens in drinking water and wastewater, and explores methods for water purification.

- **Air microbiology**

This field studies microorganisms present in the air, including their prevalence and modes of transmission. Its significance lies in understanding airborne diseases caused by microbes carried in the air or attached to dust particles.

- **Food microbiology**

This discipline involves the study of microorganisms in food, examining both their positive and negative effects on food products. It ensures food safety by monitoring methods of processing, cooking, storing, serving, and preserving food from microbial spoilage.

- **Industrial microbiology**

This field focuses on microorganisms used in industry for the production of antibiotics, enzymes, organic acids, vitamins, alcohols, and other compounds, with an emphasis on maximizing production volume while minimizing costs.

- **Agricultural microbiology**

This area studies microorganisms that impact agriculture both positively and negatively.

- **Medical Microbiology**

This field is concerned with disease-causing microorganisms, focusing on their identification through diagnosis and understanding treatment methods.

2. Historical View of Microbiology

2.1. Early observations and Islamic contributions

The discovery of microorganisms was an indirect process that began with observations of fermentation in dough and dairy products. Initially, the significance of microbial activity in disease development was not understood. Due to the limited resolving power of the human eye, which cannot detect objects smaller than 0.1 mm in diameter, early recognition of microbes was impossible.

Several Islamic scholars made significant contributions to the early understanding of infectious diseases (Figure 1):

- **Abu Bakr al-Razi (865-925):** He was the first to distinguish between smallpox and measles, which helped advance the understanding of infectious diseases.
- **Abu l-Qasim al-Zahrawi (936-1013):** He provided detailed accounts of boils, along with analyses of symptoms and treatment options for diseases caused by microorganisms.
- **Ibn Sina (980-1037):** He elucidated the modes of transmission for certain infectious diseases, including smallpox and measles. He postulated that these diseases are transmitted via microorganisms present in water and air, stating: "*Water contains minute organisms that are invisible to the naked eye and are the causative agents of certain diseases*." This assertion was later corroborated following the invention of the microscope.

(a) (b) (c)

Figure 1. Various Islamic scholars contributed to the early understanding of infectious diseases. **(a):** Abu Bakr al-Razi (865-925); **(b):** Abu l-Qasim al-Zahrawi (936-1013); **(c):** Ibn Sina (980-1037).

2.2. The birth of modern microbiology

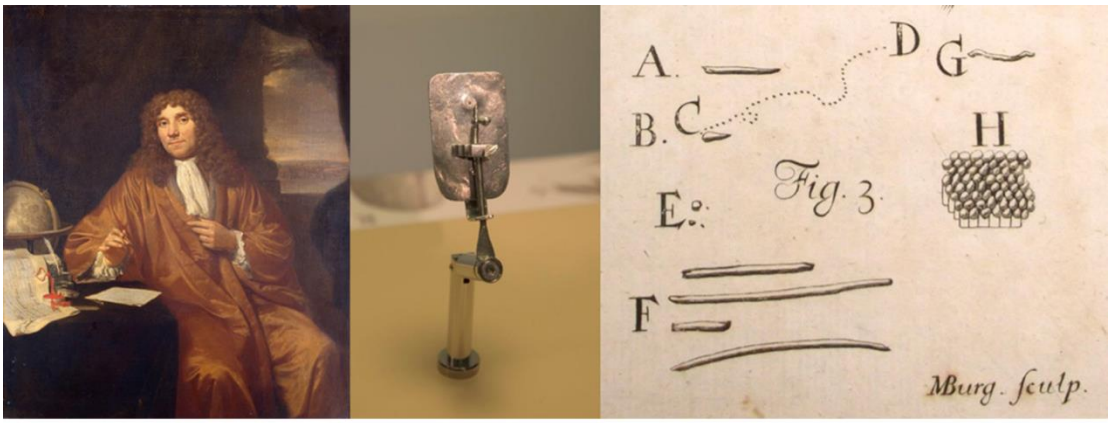
2.2.1. Antonie van Leeuwenhoek (1632-1723)

Leeuwenhoek, a Dutch textile merchant, used magnifying lenses to examine fabric threads. His passion for science led him to develop a simple microscope with a single lens that allowed him to observe microscopic organisms (Figure 2).

In 1676, Leeuwenhoek made the first observation of microorganisms in a drop of water, referring to them as "*Animalcules*." Using microscopes, he constructed himself, he achieved magnification of up to 300 times. He provided comprehensive descriptions of various microorganisms, including bacteria, yeasts, algae, protozoa, sperm, and blood cells.

Leeuwenhoek observed and accurately described three distinct bacterial forms: rod-shaped, spherical, and spiral (Figure 2). He documented his findings in letters to the Royal Society in London beginning in 1679, constituting the first known account of what are now called microorganisms.

Although Leeuwenhoek manufactured approximately 400 basic microscopes, their limited magnification power (up to 300 times) meant that further studies of microorganisms were not possible until the development of more sophisticated microscopes a century after his death.



(a) (b) (c)

Figure 2. Birth of Microbiology. **(a):** Antonie van Leeuwenhoek (1632-1723); **(b):** Microscope invented by Leeuwenhoek; (c): The three distinct bacterial forms described by Leeuwenhoek.

2.2.2. The Spontaneous generation theory

Following the discovery of microbes, scientists began investigating their origins. Two schools of thought emerged:

- **Spontaneous Generation Theory:** Proposed that microorganisms could arise spontaneously from non-living matter.
- **Germ Theory:** Posited that microbes must have a living origin, namely germs or grains found in the air, and that they do not arise from organic matter but only use it for growth.

This controversy was resolved through a series of experiments by several scientists:

a) Lazzaro Spallanzani's experiments

The Italian scientist Spallanzani (1729-1799) was the first to provide definitive evidence against spontaneous generation in 1765 (Figure 3). His experiments demonstrated that boiling organic broths for an adequate duration while tightly sealing them to prevent the ingress of air and dust effectively inhibited microbial growth.

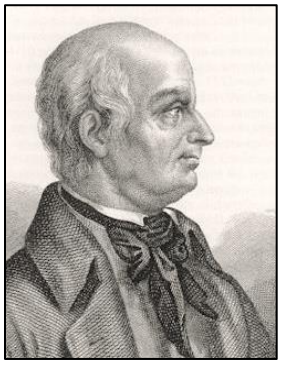


Figure 3. The Italian scientist Lazzaro Spallanzani (1729-1799).

However, the lack of knowledge at that time about heat-resistant spores led to the growth of microbes in some well-boiled and sealed liquids, which temporarily supported the theory of spontaneous generation. Spallanzani proposed that the spoilage of some liquids after boiling occurred because lids were not tightly sealed, allowing the entry of air laden with microorganisms.

b) Louis Pasteur's experiments

The various experiments conducted by the French scientist Louis Pasteur (1822-1895) and published in 1863 ultimately disproved the theory of spontaneous generation. Pasteur's experiments confirmed the presence of microbes in air and dust particles and demonstrated that keeping microbes away from sterile liquids prevented spoilage.

In his famous experiment, Pasteur devised a flask with a long, narrow, convoluted tube that prevented dust particles and microbes from entering while allowing air to flow. When he boiled meat broth in this flask and left it exposed to air through the tube, no microbial growth occurred. This was because microorganisms attached to dust particles were deposited on the inner surface of the winding tube, allowing only fresh air to enter.

In contrast, when Pasteur boiled meat broth in a straight-tube flask and exposed it to air, microbial growth was observed. This demonstrated that microbes associated with dust particles from the external environment were responsible for proliferation in the liquid rather than spontaneous generation (Figure 4).

Pasteur's experiments conclusively showed that microorganisms cannot arise from sterile organic matter isolated from air; they only develop when organic matter comes into contact with air carrying microbes.

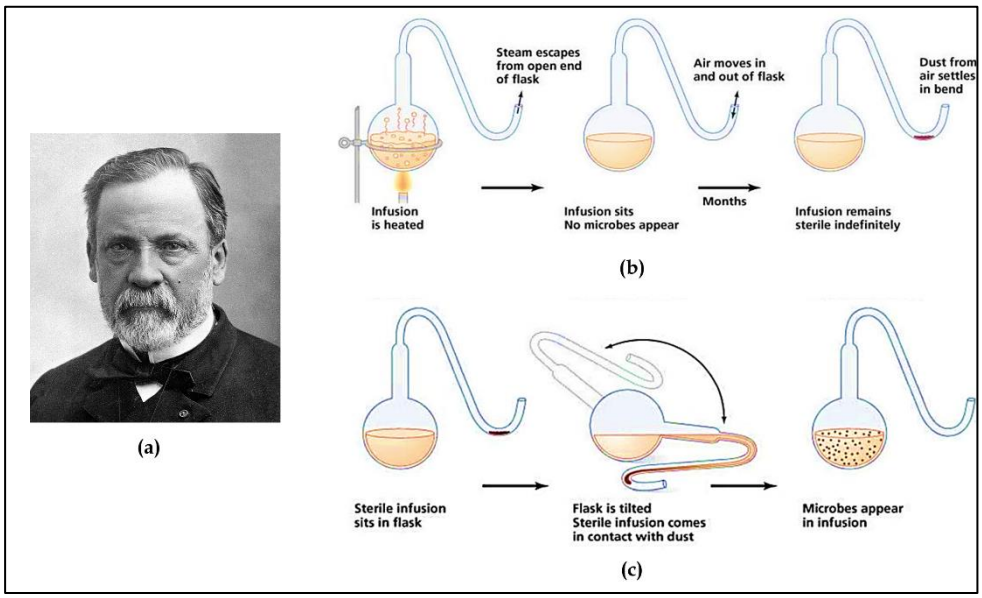


Figure 4. Louis Pasteur and the disproving of the spontaneous generation theory.
(a): Louis Pasteur (1822-1895); **(b)** and **(c):** Pasteur's experiments.

c) John Tyndall's experiments

The research conducted by John Tyndall (1820-1893) (Figure 5) revealed the existence of heat-resistant spores that could survive boiling. He demonstrated that microbial growth in covered (sterile) boiled liquids was attributable to these spores. By eliminating the spores, liquids could be preserved indefinitely without microbial growth. Tyndall established the process of intermittent steam sterilization to kill spores, a method later named "Tyndallization" in his honor. These findings completely disproved the theory of spontaneous generation.

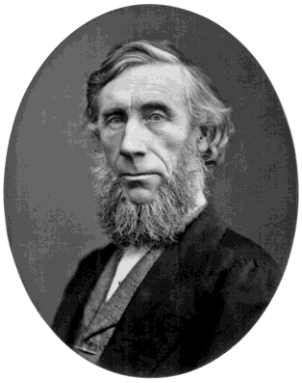


Figure 5. John Tyndall (1820-1893).

2.3. Evolution of microbiology

2.3.1. Louis Pasteur's Contributions

Beyond disproving spontaneous generation, Pasteur made numerous other contributions to microbiology:

- He confirmed that microorganisms play a pivotal role in fermentation, showing that the chemical changes in this process are mostly caused by microbes.
- He isolated and identified yeast as a principal actor in alcoholic fermentation.
- He developed the "pasteurization process" to eliminate undesired microbes without compromising beverage quality by heating wine for 30 minutes at 62.8°C (below boiling point) and then cooling it.
- He introduced the terms "anaerobic" for bacteria that grow without oxygen, "aerobic" for those active in the presence of oxygen, and "facultative" for those active in either condition.
- He pioneered the use of the autoclave for sterilization in microbiological research and used dry heat sterilization in ovens for glassware.
- In 1865, he showed that silkworm disease was caused by microorganisms in eggs and demonstrated that infection could be prevented by excluding diseased worms.
- He developed the concept of vaccination by showing that weakened forms of bacteria could stimulate antibody production and protect against future infection.
- He created attenuated vaccines, including one for rabies, demonstrating their value in preventing disease.

2.3.2. Robert Koch's contributions

Robert Koch (1843-1910) (Figure 6) advanced microbiology by establishing groundwork for microbiological preparations that facilitated the study and examination of germs.

Figure 6. Robert Koch (1843-1910).

His most important contributions were:

- He successfully stained bacteria on glass slides, prepared culture media, and solidified culture media using agents like gelatin to obtain colonies on a solid medium.

- These techniques enabled the isolation and purification of bacteria and the production of pure cultures.
- Using these methods, Koch isolated *Bacillus anthracis* (the anthrax-causing bacteria) in 1876.
- In 1882, he isolated *Mycobacterium tuberculosis*, the bacteria responsible for tuberculosis.
- In 1883, he identified Vibrio cholerae, the bacterium responsible for cholera.

2.3.3. Dmitri Iwanowski's contributions

In 1892, the Russian scientist Iwanowski (1864-1920) (Figure 7) isolated the causal agent of tobacco mosaic disease from an extract of diseased plants by passing it through bacterial filters. This led him to conclude that there are submicroscopic bodies, smaller than all known microorganisms, capable of causing infection – what we now call viruses.

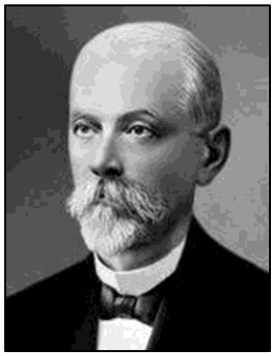


Figure 7. The Russian scientist Dmitri Iwanowski (1864-1920).

2.3.4. Alexander Fleming's contributions

The 20th century saw significant advancements in microbiology. In 1929, Alexander Fleming (1881-1955) discovered penicillin, an antibiotic that inhibits bacterial growth (Figure 8). This discovery led to the development of other antibiotics, and the antibiotic industry developed and was used in chemotherapy from 1940, helping save many people with infectious diseases.

The historical development of microbiology has made significant contributions to our understanding of the world around us. Technological advancements, particularly the advent of the electron microscope, have greatly enriched our knowledge in this field.

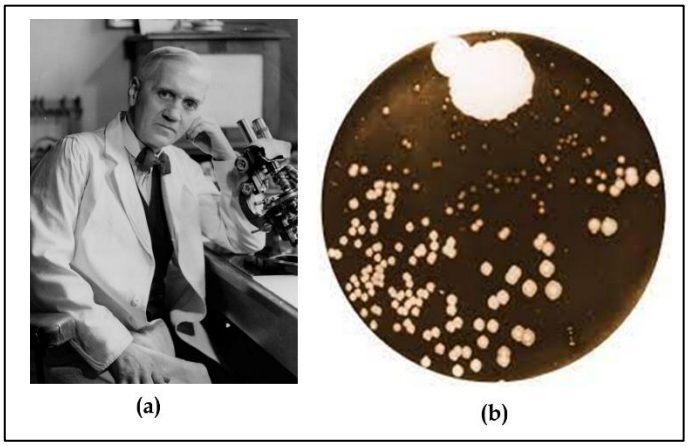


Figure 8. Fleming's contributions. **(a):** Alexander Fleming (1881-1955); **(b):** Penicillin discovery by Fleming (1929).

Chapter I

The Microbial World

Chapter I. The Microbial World

Microbiology is the study of microorganisms, which are microscopic living organisms that cannot be observed with the naked eye. This field encompasses a vast diversity of life forms, including bacteria, archaea, fungi, algae, protozoa, and viruses. Despite their microscopic size, these organisms play crucial roles in ecosystems, human health, and biotechnology.

Microbiology is one of the most applied biological sciences that emerged as a true science in the latter part of the 19th century. It is the study of microorganisms - living entities that are typically too small to be clearly perceived by the unaided human eye. By definition, microorganisms are living organisms with a diameter of 1 mm or less, most measuring only a few thousandths of a millimeter, requiring microscopes for proper observation and study.

1. Historical Development of Microbial Classification

1.1. Two-kingdom system (Earliest classification system)

The systematic classification of living organisms began with Swedish botanist Carl Linnaeus (1707-1778), who in 1735 established a taxonomic classification dividing living organisms into two kingdoms: Animalia and Plantae (Figure 9). This binary system introduced the fundamental binomial nomenclature system (genus and species) that remains essential to modern taxonomy. However, it proved inadequate as scientists discovered microorganisms with characteristics that didn't fit neatly into either kingdom.

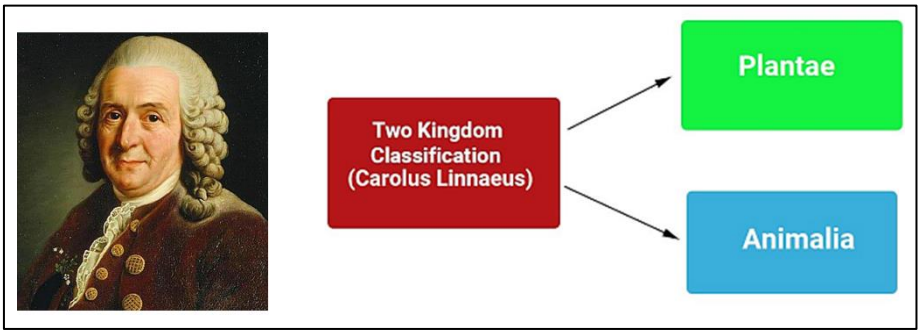


Figure 9. The two-kingdom classification by Carl Linnaeus.

In 1857, Swiss botanist Karl Nageli (1817-1891) proposed classifying bacteria and fungi within the Plantae kingdom. However, the diverse characteristics of microorganisms—some resembling plants, others animals, and many exhibiting unique properties—necessitated a more comprehensive classification system.

1.2. Three-kingdom system

In 1866, the German scientist Ernst Haeckel (1834-1919) proposed creating a third kingdom called Protista to accommodate microorganisms that didn't fit well within the plant or animal kingdoms. This three-kingdom system classified living beings based on unicellularity and

multicellularity. Haeckel's Protista initially included all microscopic organisms, including bacteria, protozoa, fungi, and algae, essentially grouping all organisms lacking tissue differentiation.

1.3. Five-kingdom system

A significant advancement came in 1959 when American ecologist Robert Whittaker (1920-1980) first proposed a five-kingdom classification system, which he refined in a 1969 publication. This system was based on cellular organization and nutritional patterns: Monera included prokaryotic organisms (bacteria, cyanobacteria), Protista included unicellular eukaryotes (protozoa, unicellular algae), Fungi included heterotrophic organisms with cell walls containing chitin, Plantae included multicellular photosynthetic organisms with cellulose cell walls, and Animalia included multicellular heterotrophic organisms without cell walls (Figure 10).

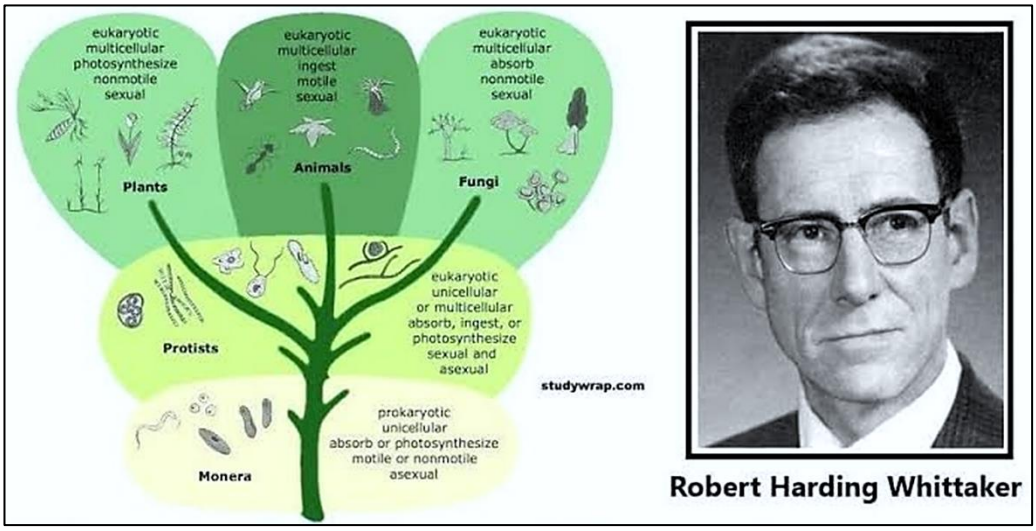


Figure 10. The five-kingdom classification by Robert Whittaker.

1.4. Six-kingdom system

In 1977, American microbiologist Carl Woese (1928-2012) revolutionized microbial classification by proposing the division of the Kingdom Monera into two distinct kingdoms: "Bacteria" (Eubacteria) and "Archaea." This work was based on a comparative analysis of ribosomal RNA sequences, which revealed that these two groups of prokaryotes were as different from each other as they were from eukaryotes.

1.5. Modern three-domain system

By 1990, Woese, along with O. Kandler and M.L. Wheelis, introduced a new classification level designated as "Domain," which sits above the kingdom level. This modern classification system divides all living organisms into three domains: domain Bacteria (Eubacteria), domain Archaea, and domain Eukarya (Eukaryota) (Figure 11).

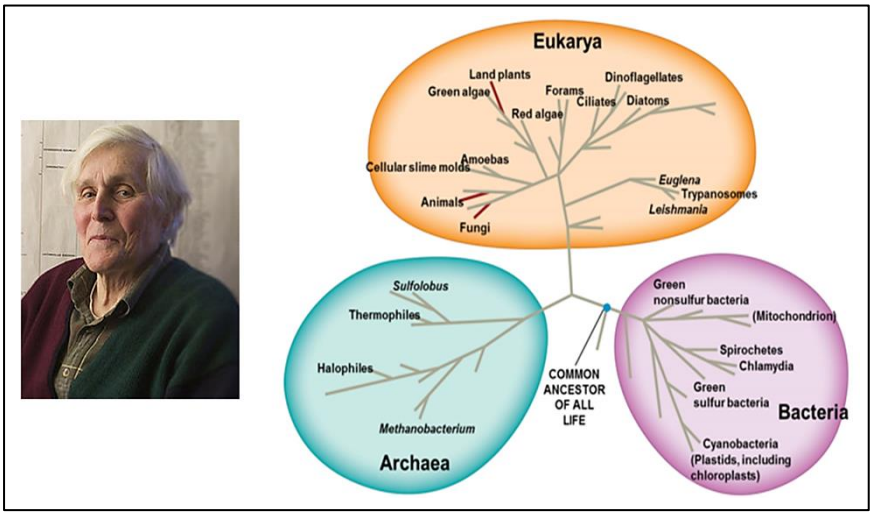


Figure 11. The three domain classifications by Carl Woese.

This three-domain system is now the accepted standard in biological classification and reflects our understanding of the fundamental differences between these groups at the cellular and molecular levels. The key differences include cell wall composition, membrane lipids, RNA polymerase structure, and numerous other biochemical and genetic characteristics that justify their separation into distinct evolutionary lineages.

3. Cellular Organization of Microorganisms

3.1. Prokaryotic versus Eukaryotic Cells

One of the most fundamental distinctions in microbiology is between prokaryotic and eukaryotic cells. This classification, proposed by French biologist Edouard Chatton (1883-1947) in 1937, divides cells based on their internal organization.

3.1.1. Prokaryotic Cells

Prokaryotic cells (bacteria and archaea) represent the simplest form of cellular organization (Figure 12). Key characteristics include:

- No true nucleus; genetic material (DNA) is freely distributed in the cytoplasm in a region called the nucleoid.
- Genetic material exists as a single circular chromosome.
- May contain small cytoplasmic plasmids (cyclic DNA molecules carrying additional genes).
- No membrane-bound organelles.
- Cell division occurs through binary fission rather than mitosis.
- Cell walls with distinctive composition (often containing peptidoglycan in bacteria).
- Cell size typically ranging from 0.2-2.0 µm in diameter.

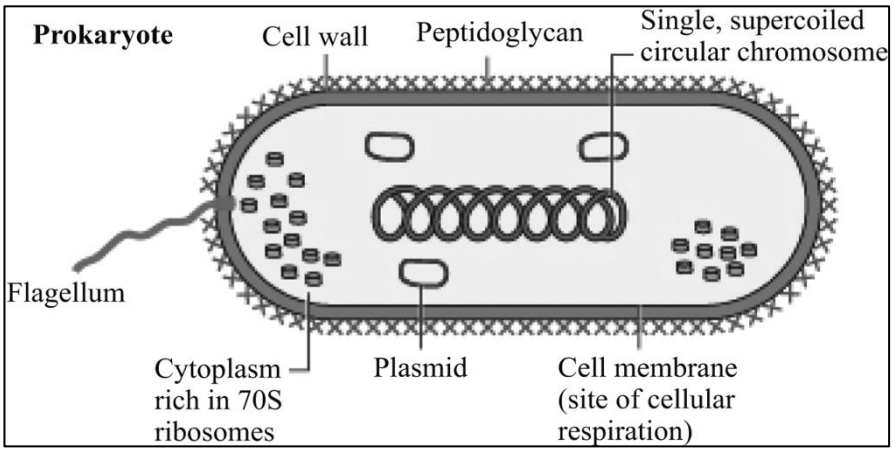


Figure 12. Prokaryotic cell.

3.1.2. Eukaryotic cells

The defining characteristics of eukaryotic microorganisms (Figure 13), fungi, algae, and protozoa include:

- Genetic material enclosed within a true nucleus surrounded by a nuclear membrane
- DNA organized into multiple linear chromosomes.
- Chromosomes undergo replication through mitosis.
- Chromosomes comprise alkaline proteins called histones.
- Contain numerous membrane-bound organelles (mitochondria, chloroplasts, Golgi apparatus, endoplasmic reticulum, lysosomes).
- Larger size, typically 10-100 µm in diameter.
- More complex internal structure and organization.

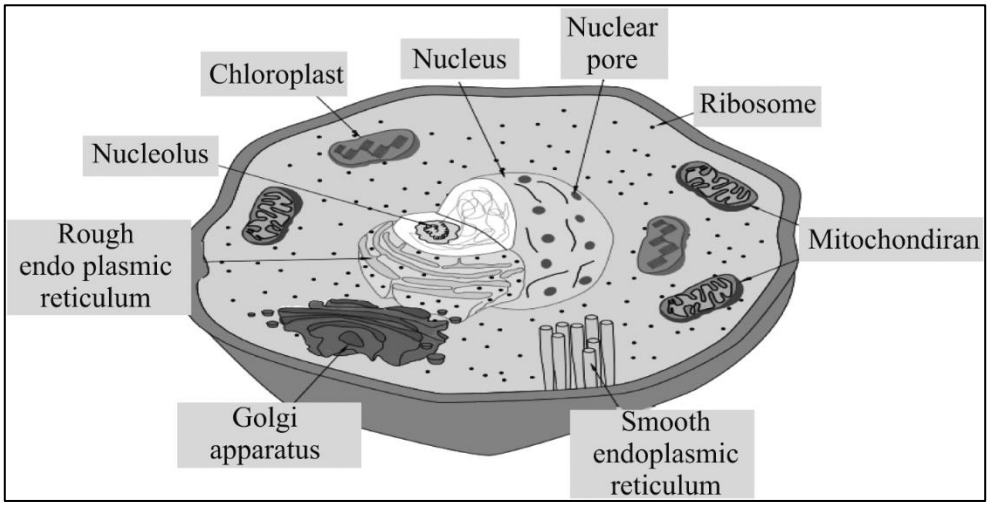


Figure 13. Eukaryotic cell.

The following table summarizes the key differences between prokaryotic and eukaryotic cells.

Table 1. The comparative table: Prokaryotic versus eukaryotic cells.

Characteristics	Prokaryotes	Eukaryotes
Type of cell	Always unicellular	Unicellular and multicellular
Cell size	From 0.2 to 2.0 µm in diameter	From 10 to 100 µm in diameter
Cell wall	Usually present	Can be present or absent
Cell wall composition	Often contains peptidoglycan	When present, contains cellulose (plants) or chitin (fungi)
Nucleus	Absent, instead DNA present in nucleoid region	True nucleus with nuclear membrane
Chromosome structure	Single circular chromosome	Multiple linear chromosomes with histones
Cell division	Binary fission	Mitosis
Membrane-bound organelles	Absent	Present (mitochondria, chloroplasts, Golgi apparatus, endoplasmic reticulum, lysosomes)
Ribosomes	Small ribosomes (70S)	Large ribosomes (80S)
Plasmids	Present	Very rarely found in eukaryotes
Flagella	Present, smaller in size	Present, larger in size
Reproduction	Asexual	Both sexual and asexual

This fundamental difference in cellular organization reflects the evolutionary divergence that occurred billions of years ago and underlies many of the physiological and metabolic differences between these groups of microorganisms.

3.2. Types of cellular organization of microorganisms

Microorganisms exhibit diverse forms of cellular organization that can be categorized into three main types:

3.2.1. Single-cell microorganisms

The majority of microorganisms, including bacteria, protozoa, yeasts, and many algae, are unicellular. Each individual is composed of a single self-sufficient cell that forms a complete and autonomous organism capable of performing all life functions, including nutrition, growth, and reproduction. Examples include yeast, amoeba, and paramecium.

3.2.2. Multicellular microorganisms

Some microorganisms exhibit multicellular organization, where multiple cells function together as a single organism. The most prominent representatives of this group are filamentous fungi, certain

algae, and a small number of bacteria, including cyanobacteria. These organisms are composed of multiple cells that may be identical in function and structure

3.2.3. Coenocytic Microorganisms

Coenocytic microorganisms consist of a large cytoplasm containing many nuclei with no barriers between them (Figure 14). They are primarily represented by primitive fungi and a few algae. This unique cellular organization allows for rapid growth and resource sharing across the organism.

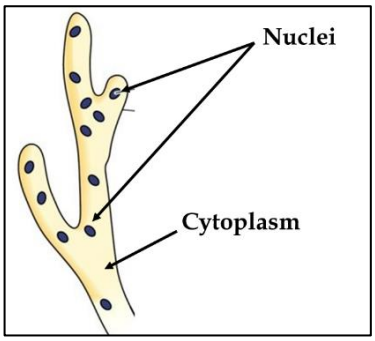


Figure 14. Coenocytic cellular organization.

4. Domain Bacteria

Bacteria represent one of the three domains of life and are prokaryotic microorganisms characterized by their simple cellular organization. They are ubiquitous, found in virtually every environment on Earth, from deep-sea hydrothermal vents to the human gut. The domain of Bacteria comprises several groups, the most important of which are the following groups: Gram-positive bacteria, Gram-negative bacteria, Mycoplasma, and Cyanobacteria.

4.1. Gram-positive and Gram-negative bacteria

Based on the structure of the cell wall and its susceptibility to Gram staining, bacteria are classified into two main types: Gram-positive and Gram-negative bacteria (Figure 15). Detailed bacterial structure will be explored in Chapter Two.

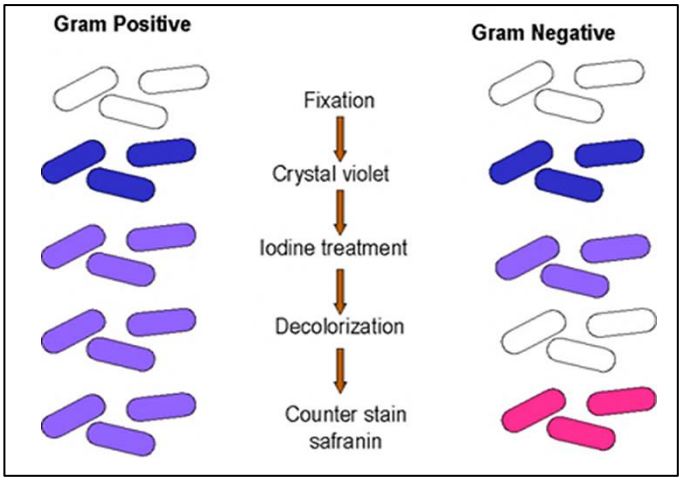


Figure 15. The Gram staining technique.

4.1.1. Gram-positive bacteria

Gram-positive bacteria retain the violet coloration during Gram staining due to their thick peptidoglycan layer (Figure 16). Their cell wall contains multiple layers of peptidoglycan (up to 90% of wall components), teichoic acid, and minimal or no protein content.

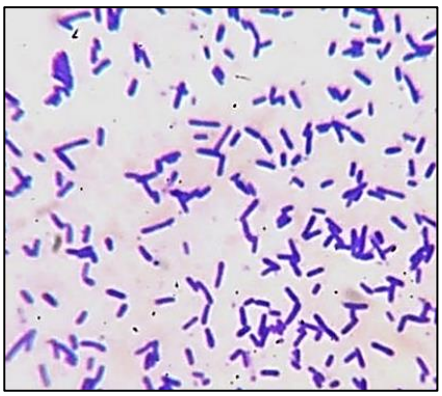


Figure 16. Microscopic observation of a Gram-negative bacteria.

The important groups of Gram-positive bacteria include:

- **Cocci**: Non-motile, non-spore-forming bacteria that can be either pathogenic (e.g., *Staphylococcus aureus*, *S. pneumoniae*) or non-pathogenic.
- **Spore-forming bacilli**: These bacteria form spores that are resistant to harsh environmental conditions, including *Bacillus* and *Clostridium*. Some cause diseases in humans, animals, and plants.
- **Non-spore-forming bacilli**: Aerobes or facultative anaerobes, such as *Corynebacterium* and *Listeria*.
- **Actinomycetes**: Morphologically similar to fungi, they can form mycelium. Some species are parasitic, causing diseases in plants, animals, and humans. Examples include *M. tuberculosis* (tuberculosis) and Streptomyces, which produces antibiotics like Streptomycin.

4.1.2. Gram-negative bacteria

Gram-negative bacteria lose the violet coloration during Gram staining and appear pink when counterstained with Safranin or fuchsin (Figure 17). Their cell wall comprises three layers: a thin mucopeptide layer (peptidoglycan), lipopolysaccharides (which rapidly dissolve in alcohol during staining), and lipoproteins.

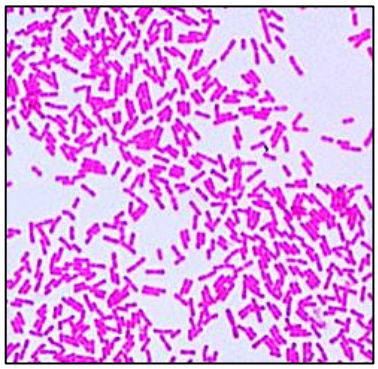


Figure 17. Microscopic observation of a Gram-negative bacteria.

Gram-negative bacteria can be classified based on their oxygen requirements:

- **Facultative anaerobic bacilli**: These include intestinal bacteria that live in the digestive tract of animals, such as Escherichia coli.
- **Obligate aerobes**: These bacteria require oxygen for growth and include many nitrogen-fixing species like *Rhizobium* and *Azotobacter*.
- **Microaerophilic bacteria**: These require oxygen at lower concentrations than is present in the atmosphere and are sensitive to higher oxygen levels, such as Helicobacter pylori.
- **Obligate intracellular bacteria**: Including the *Rickettsia* group and the *Chlamydia* group, these bacteria can only reproduce within host cells.
- **Spirochetes**: Spiral-shaped bacteria, most of which are parasitic and cause disease, including *Treponema pallidum* (syphilis).

4.1.3. Mycoplasma

Mycoplasma represents a unique group of bacteria characterized by:

- Lack of a cell wall, making them resistant to many antibiotics that target cell wall synthesis.
- Flexible shape due to the absence of a rigid cell wall.
- Small size (0.2-0.3 µm), capable of passing through standard bacterial filters.
- Parasitic or saprophytic lifestyle, infecting humans, animals, and plants.
- Fastidious growth requirements make them difficult to culture.

4.1.4. Cyanobacteria

Formerly known as blue-green algae, cyanobacteria have been regrouped with bacteria due to their prokaryotic nature (Figure 18). Key characteristics include:

- Photosynthetic (oxygen-producing) capability similar to plants.
- Gram-negative cell wall structure.
- Contain a blue pigment (Phycocyanin).
- Found in diverse environments, including seas, oceans, fresh water, hot water, and soil.
- Exhibit various cellular organizations from unicellular to complex multicellular forms.

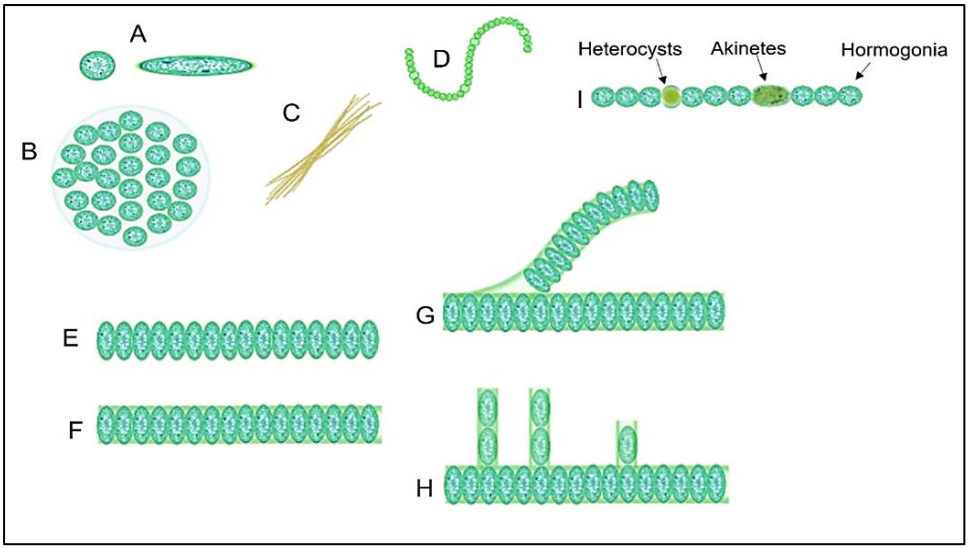


Figure 18. Different forms of cyanobacteria. **(A):** spherical and ovoid unicellular; **(B):** colonial; **(C):** filamentous; **(D):** spiral; **(E):** unsheathed trichome; **(F):** sheathed trichome; **(G):** false branching; **(H):** true branching; **(I):** different cell types in filamentous cyanobacteria.

5. Domain Archaea

Archaea constitute a distinct domain of single-celled, prokaryotic microorganisms that differ significantly from bacteria and eukaryotes. Initially classified as bacteria, they were recognized as a separate domain based on molecular studies, particularly rRNA sequence analysis conducted by Carl Woese in the late 1970s.

The general characteristics of Archaea include:

- Prokaryotic cells lacking membrane-bound organelles
- Cell walls devoid of peptidoglycan, instead containing other complex structures like S-layer and pseudomurein.
- Cell membranes contain ether-linked lipids (rather than ester-linked lipids found in bacteria), enhancing stability in extreme environments.
- Archaeal ribosomes are more similar to eukaryotic ribosomes than bacterial ribosomes.
- DNA present in covalently closed circular form, similar to bacteria.
- RNA polymerases are more complex (8-12 subunits) than bacterial RNA polymerases (4 subunits).
- Promoter structure contains TATA box similar to eukaryotes.
- Insensitive to many antibiotics that affect bacteria (chloramphenicol, streptomycin, kanamycin).
- Ability to flourish in diverse habitats, including extreme environments.

Archaea can be classified into different groups based on their physiological characteristics and habitats:

a) Methanogens

- Produce methane (CH_4) as a byproduct of their metabolism.

- Obligate anaerobes that reduce carbon dioxide, hydrogen, acetate, or methanol.
- Found in anaerobic environments such as swamps, the digestive tracts of ruminants, the sediments of lakes and oceans, and landfills.
- Include genera such as *Methanococcus*, *Methanobrevibacter*, *Methanosarcina*, and *Methanogenium*.

b) Halophiles

- Require high salt concentrations for growth and reproduction.
- Can be both aerobic and anaerobic, depending on the specific group.
- Thrive in highly saline environments such as the Dead Sea (salinity of about 30%), salt lakes, and salt ponds.
- Examples include *Halobacterium*, *Haloferax*, and *Halococcus*.

c) Thermophiles

- Prefer high-temperature environments, often above 45°C.
- Found in hot springs, hydrothermal vents, volcanic environments, geothermal areas, hot acidic springs, hot acidic soil, and sulfur springs.
- Examples include *Thermoproteus* (thriving at 70-100°C), *Pyrococcus* (optimal growth temperatures often exceeding 100°C), *Sulfolobus* (thriving at pH 2.0-4.5 and temperatures of 70-80°C), and *Thermoplasma* (optimal growth at 55-65°C and pH 2.0-4.0).
- Some thermophiles are also sulfur-reducing archaea that convert sulfur compounds to hydrogen sulfide.
- Most thermophilic archaea are anaerobic.

The unique characteristics of Archaea, particularly their ability to thrive in extreme environments, make them valuable subjects for studying the limits of life and for potential biotechnological applications.

The following table summarizes the key differences between Bacteria and Archaea.

Table 2. The comparative table: Bacteria versus Archaea.

Archaea	**Bacteria (Eubacteria)**
Simple in their organization	More complex than Archaea
Found in extreme environments	Found everywhere on earth
Sphere, rod, spiral, flat or square-shaped	Cocci, bacilli, vibrio, filament or spirochetes in shaped
Cell wall is composed of pseudopeptidoglycan	Cell wall is composed of peptidoglycan with muramic acid
Intron are absent	Introns are present
Exhibit neither glycolysis nor Kreb's cycle	Exhibit both glycolysis and Kreb's cycle
Has three types: methanogens, halophiles, and thermophiles	Has two types: Gram-positive and Gram-negative

Table 2. Continued.

Archaea	Bacteria (Eubacteria)
Asexual reproduction: methods like binary fission, budding and fragmentation are used.	Capable of producing spores to remain dormant during unfavorable conditions.
RNA polymerase consists of a complex subunit pattern.	RNA polymerase consists of a simple subunit pattern.
Membrane lipids are ether-linked, branched, aliphatic chains containing D-glycerol phosphate.	Membrane lipids are ester-linked, branched, straight chains of fatty acids containing L-glycerol phosphate.

6. Eukaryotic Microorganisms

Eukaryotic microorganisms represent a diverse group characterized by their complex cellular organization. They include fungi, algae, and protozoa, each with distinct characteristics and ecological roles.

6.1. Microscopic fungi (molds and yeasts)

Fungi are eukaryotic organisms that lack chlorophyll and obtain nutrients through absorption. They play crucial roles in decomposition, symbiotic relationships, and as pathogens.

6.1.1. General characteristics of microscopic fungi

- **Cellular structure:** Eukaryotic cells with membrane-bound nuclei and organelles. Cell walls are primarily composed of chitin, providing structural support (Figure 19). They lack chloroplasts and chlorophyll, unable to perform photosynthesis.

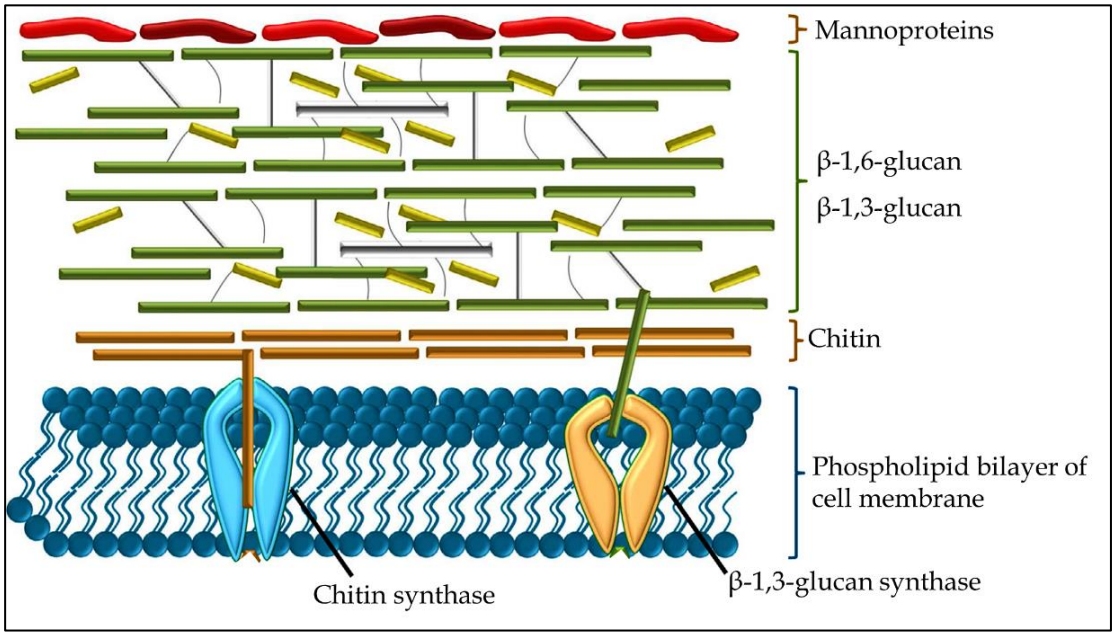


Figure 19. Schematic overview of fungal cell wall composition.

- **Morphology:** Most fungi are filamentous, consisting of hyphae that form a mycelium. Yeasts are typically unicellular, though some species can form multicellular pseudohyphae (Figure 20). Some fungi exhibit dimorphism, existing in both yeast and hyphal forms depending on environmental conditions.

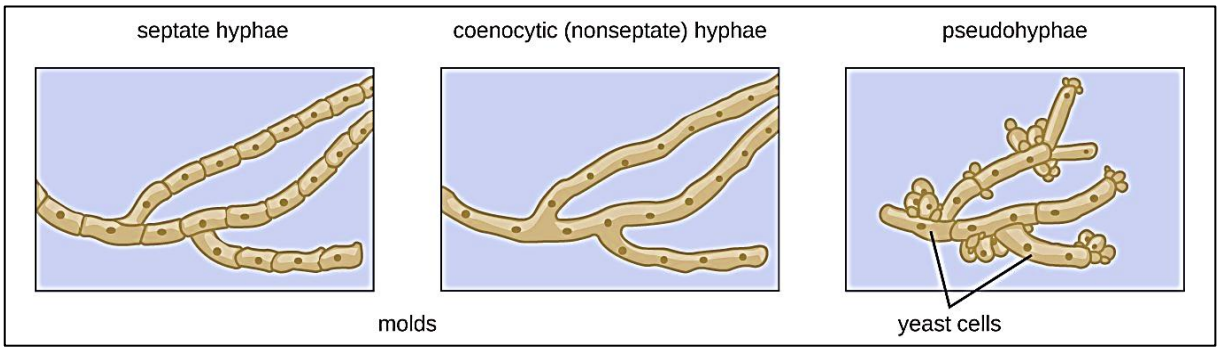


Figure 20. Hyphae of fungi.

- **Reproduction:** Molds reproduce both sexually and asexually through fragmentation or spore formation. Yeasts primarily reproduce asexually through budding or fission. Sexual reproduction occurs via specialized structures in some species.
- **Growth conditions:** Most fungi grow in moderate temperatures (20-30°C). They require high humidity or moisture and prefer slightly acidic conditions (pH 4-6). Most fungi are obligate aerobes.

6.1.2. Mode of nutrition in fungi

Based on their nutritional strategies, fungi can be classified into:

- **Saprophytic fungi:** Obtain nutrients from dead and decaying organic matter and are essential decomposers in ecosystems. They play crucial roles in nutrient cycling.
- **Parasitic fungi:** Obtain nutrition from living organisms, often causing harm. They include plant pathogens (obligate and facultative parasites), animal and human pathogens like *Candida* (Figure 21) and *Aspergillus*.
- **Symbiotic fungi:** Form mutually beneficial relationships with other organisms. They include mycorrhizae (associations with plant roots) and lichens (symbiotic association between fungi and algae or cyanobacteria).

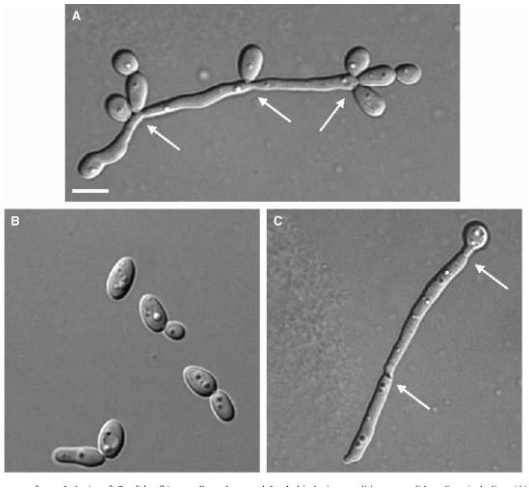


Figure 21. Morphologies of human pathogen *Candida albicans.* **(A):** Branched pseudohyphal structure with lateral buds; **(B):** Yeast cells; **(C):** Highly elongated pseudohyphal filament. Arrows point to constrictions at septal sites. The scale bar is 10 µm.

6.1.3. Classification of fungi

Microscopic fungi are classified into several groups based on their morphological characteristics and modes of reproduction:

a) Ascomycetes (Ascomycota) :

- Largest and most diverse group of fungi.
- Characterized by the production of ascospores within asci.
- Cell walls contain chitin and β-glucans.
- Typically have septate hyphae.
- Reproduce both sexually and asexually.
- Include yeasts like *Saccharomyces cerevisiae*, molds like *Penicillium* and *Aspergillus* (Figure 22).

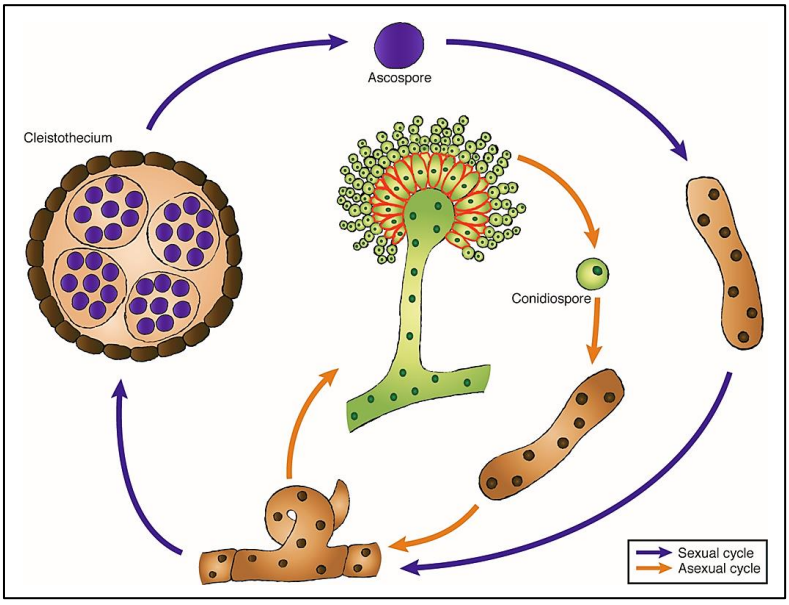


Figure 22. Life cycle of *Aspergillus*.

b) Zygomycetes (Zygomycota):

- Have coenocytic hyphae (lacking septa).
- Cell walls contain chitin and chitosan.
- Many are saprotrophic, like *Rhizopus* (bread mold).
- Reproduce asexually through sporangiospores.
- Sexual reproduction is characterized by zygospore formation.

c) Basidiomycetes (Basidiomycota) :

- Characterized by unique reproductive structures called basidia.
- Possess septate hyphae with specialized pores.
- Sexual reproduction involves the formation of basidiospores
- Include yeasts like *Cryptococcus* and molds like *Puccinia graminis.*

d) Deuteromycetes (Imperfect Fungi) :

- Characterized by asexual reproduction and lack of known sexual reproduction.
- Possess well-developed, branched, and septate hyphae.
- Reproduce asexually through conidia.
- Include medically important fungi like *Candida albicans*.

6.2. Microscopic algae

Microscopic algae, or microalgae, are predominantly unicellular eukaryotic organisms that perform photosynthesis. They are found in freshwater and marine environments and contribute significantly to oxygen production on Earth.

6.2.1. General characteristics of microalgae

- Predominantly unicellular eukaryotic organisms, though some form colonies or filaments.
- Perform photosynthesis, converting CO_2 and H_2O into organic compounds and O_2.
- Contribute to approximately 50% of Earth's oxygen production.
- Contain various pigments, including chlorophylls, carotenoids, and phycobiliproteins.
- Cell walls are often composed of cellulose, silica (in diatoms), or other polysaccharides.
- Exhibit different cell organization forms: unicellular, colonial, and filamentous.

6.2.2. Classification of microalgae

Microalgae are classified into several groups based on their pigmentation, cell wall composition, and other characteristics:

a) Green algae (Chlorophyta):

- Contain chlorophyll a and b, giving them a green color.
- Photosynthetic pigments similar to land plants.
- Cell walls are primarily composed of cellulose.
- Examples: *Chlorella* (Figure 23), *Chlamydomonas*.

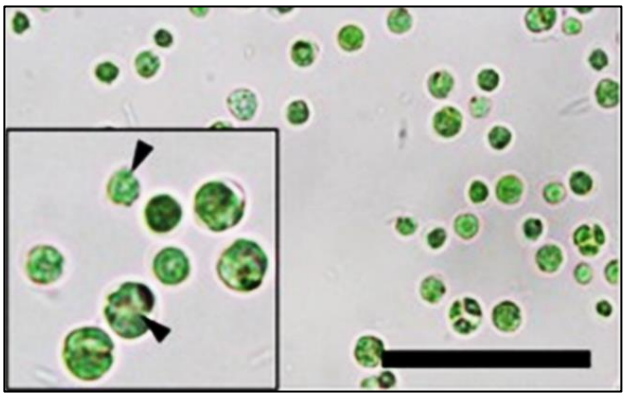


Figure 23. A microscopic image of *Chlorella vulgaris*. Scale bar: 20 µm.

b) Red algae (Rhodophyta):

- Contain chlorophyll a and phycobilins (phycoerythrin gives them a red color).
- Mostly marine species used in the food industry for products like agar and carrageenan.
- Cell walls are composed of cellulose and other polysaccharides like agar.
- Example: *Porphyridium* (Figure 24).

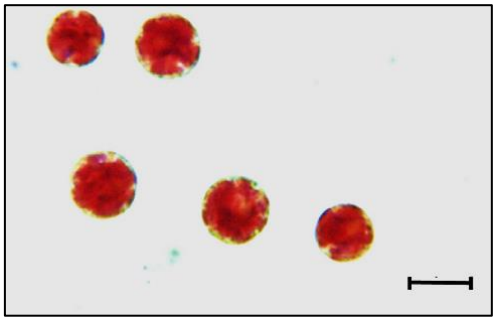


Figure 24. A microscopic image of *Porphyridium purpureum*. Scale bar: 4 µm.

c) Diatoms (Bacillariophyta):

- Cell walls are made of silica (forming a glass-like structure called a frustule).
- Contain chlorophyll a, c, and fucoxanthin (brownish pigment).
- Examples: *Thalassiosira* and *Fragilariopsis* (Figure 25).

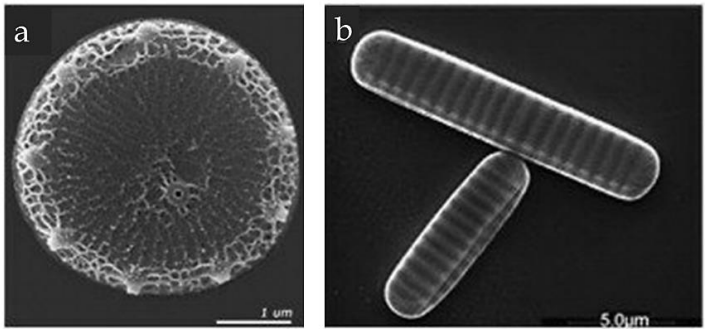


Figure 25. Some diatom species. **(a):** *Thalassiosira pseudonana* (scale bar: 1 µm); **(b):** *Fragilariopsis cylindrus* (scale bar: 5.0 µm).

d) Euglenoids (Euglenophyta):

- Contain chlorophyll a and b.
- The storage product is paramylon (carbohydrate similar to starch).
- No rigid cell wall; instead, they have a flexible pellicle made of protein strips.
- Example: *Euglena*. (Figure 26).

Figure 26. Euglena gracilis under a transmission light microscope. The cell is 80 µm long.

e) Golden Algae (Chrysophyta):

- Contain chlorophyll a and c, as well as accessory pigments like fucoxanthin.
- Golden-brown pigmentation.
- Primarily freshwater species.
- Examples: *Derepyxis* and *Chrysopyxis* (Figure 27).

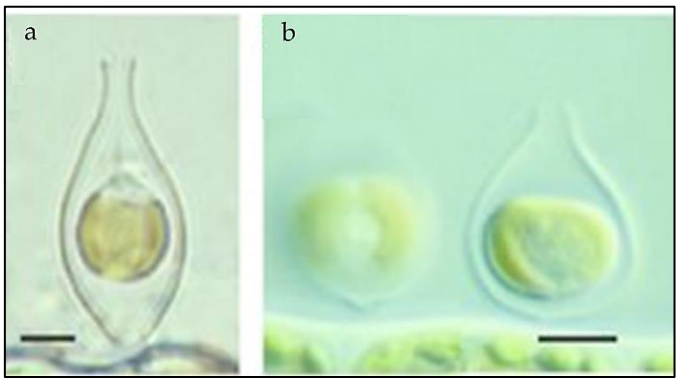


Figure 27. Images of some Chrysophytes. **(a):** *Derepyxis* sp.; **(b):** *Chrysopyxis* sp. Scale bar: 5 µm.

f) Dinoflagellates (Dinophyta):

- Contain chlorophyll a, c, and peridinin (a carotenoid).
- Cell wall composed of cellulose plates called thecae.
- Examples: *Alexandrium* and *Ceratium* (Figure 28).

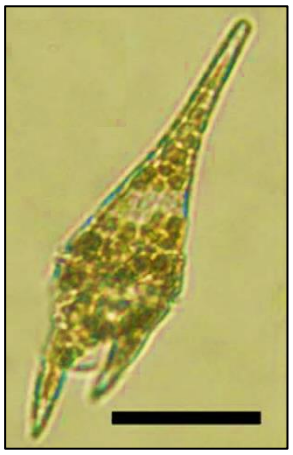


Figure 28. Cell morphology of *Ceratium furca*. Scale bar: 50 µm.

6.2.3. Reproduction in microalgae

Microalgae reproduce through both asexual and sexual methods:

- **Asexual reproduction:** The most common method, enabling rapid population growth. Binary fission is the primary method in unicellular microalgae, fragmentation in multicellular filamentous algae. Microalgae can also reproduce by spore formation, producing either zoospores (flagellated) or aplanospores (non-motile).
- **Sexual reproduction:** Occurs by conjugation, alignment of filaments and formation of conjugation tubes, or by isogamy (fusion of morphologically identical gametes) or by Anisogamy: fusion of morphologically different gametes.

6.3. Protozoa

Protozoa are unicellular eukaryotic organisms that live in aqueous environments and soil. They are typically larger and more complex than bacteria, ranging from 10-50 µm in size, though some can reach up to 1 mm.

6.3.1. General characteristics of protozoa

- Unicellular eukaryotic organisms.
- Heterotrophic nutrition, obtaining nutrients by consuming other organisms or organic matter
- Motility through various structures like cilia, flagella, or pseudopodia.
- Reproduction by binary fission, multiple fission, or sexual processes.
- Form protective cysts in unfavorable conditions
- Free-living or parasitic lifestyles.

6.3.2. Classification of protozoa

Protozoa are traditionally classified based on their locomotory structures:

a) Sarcodina (Rhizopoda):

- Move using pseudopodia (temporary extensions of the cytoplasm).

- Examples include *Amoeba* (freshwater) and *Entamoeba histolytica* (human pathogen causing amoebiasis) (Figure 29).
- Foraminifera are marine species with shells made of calcium carbonate.

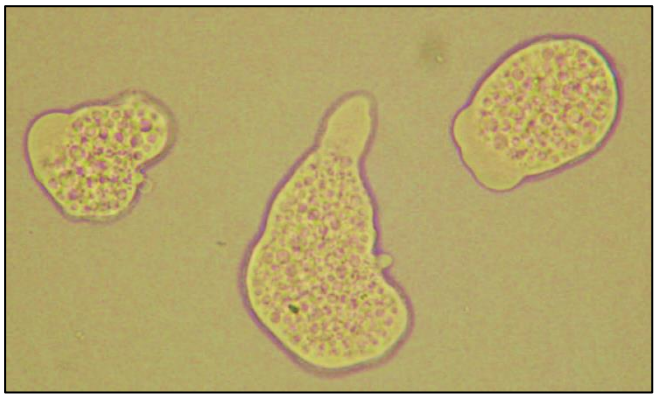


Figure 29. *Entamoeba histolytica* under microscope.

b) Mastigophora (Flagellata):

- Possess one or more flagella for movement.
- Examples include *Trypanosoma* (causing African sleeping sickness) (Figure 30), and *Trichomonas* (causing urethritis and vaginitis).

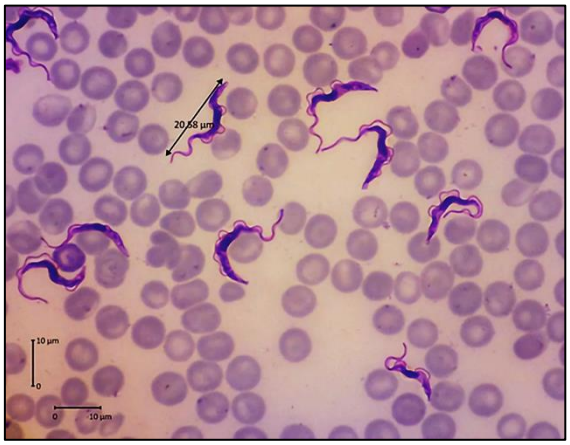


Figure 30. Morphology of *Trypanosoma evensii* observed under a light microscope (400 ×).

Scale bar: 10 µm

c) Ciliophora (Ciliata):

- Move using cilia.
- Characterized by two nuclei: a micronucleus (for reproduction) and a macronucleus (for cell functions).
- Example: *Paramecium caudatum* (Figure 31).

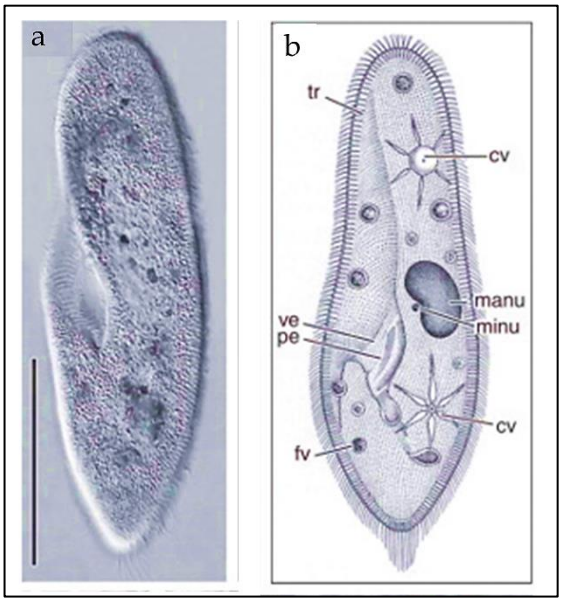


Figure 31. *Paramecium caudatum*. **(a):** Light microscopic appearance of *P. caudatum* (scale bar: 100 µm); **(b):** Drawing of *P. caudatum* illustrating light microscopic features.

d) Sporozoa (Apicomplexa):

- Lack of locomotory structures in the adult stage.
- Reproduce by spore formation.
- Examples: *Plasmodium*, *Toxoplasma gondii* (Figure 32).

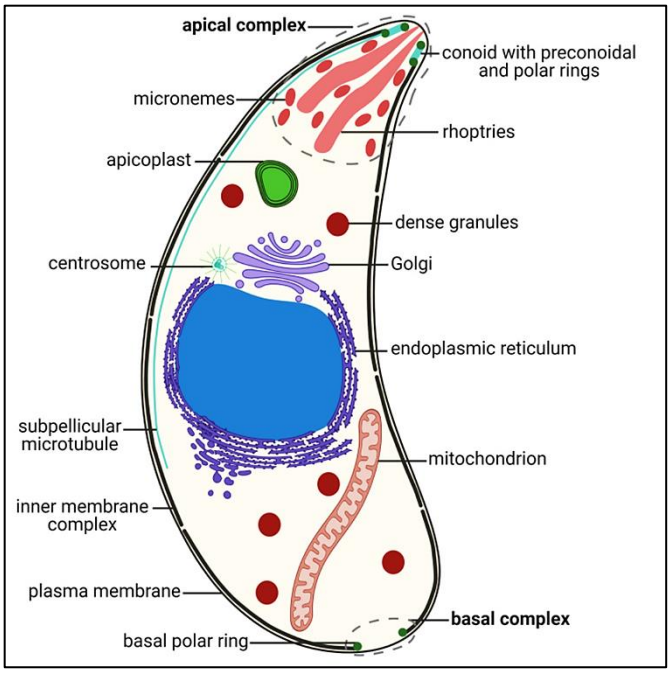


Figure 32. Morphology of *Toxoplasma gondii*.

Chapter II

Morphology and Structure of Bacteria

Chapter II. Morphology and Structure of Bacteria

Bacteria are prokaryotic microorganisms devoid of chlorophyll and are unicellular. Unlike eukaryotes, bacterial cells lack membrane-bound organelles and a true nucleus. Bacteria are among the most abundant organisms on Earth, exhibiting remarkable diversity in their morphology, structure, and function.

1. Bacterial Morphology

Bacterial morphology refers to the shape, arrangement, and size of bacterial cells, which are key characteristics for identification and classification. The bacterial cell typically measures 0.2-1.5 µm in diameter and about 3-5 µm in length, making them invisible to the naked eye and requiring microscopy for visualization.

1.1. Shapes and arrangements of bacteria

Due to the presence of a rigid cell wall, bacteria maintain a definite shape, though they vary in shape size, structure and arrangement.

1.1.1. Shapes

a) Basic shapes of bacteria

The three fundamental bacterial shapes are (Figure 33):

- **Cocci (spherical):** Derived from Greek "*kokkos*", meaning berry. Examples: *Staphylococcus, Streptococcus*.
- **Bacilli (rod-shaped):** From Latin "*baculus*" meaning rod. Examples: *Escherichia coli, Bacillus subtilis*.
- **Spiral forms:** Helically twisted cylindrical cells that can be further classified into:
  - ✓ **Vibrios:** Comma-shaped curved rods with characteristic vibratory motility. Example: *Vibrio cholerae*.
  - ✓ **Spirilla (singular spirillum):** Rigid spiral-shaped bacteria with external flagella. Example: *Spirillum volutans*.
  - ✓ **Spirochetes:** Are long and have flexible cell walls. They use internal axial filaments (endoflagella) for movement. Examples: *Treponema, Borrelia*.

The following figure represents the basic cell shapes of bacteria.

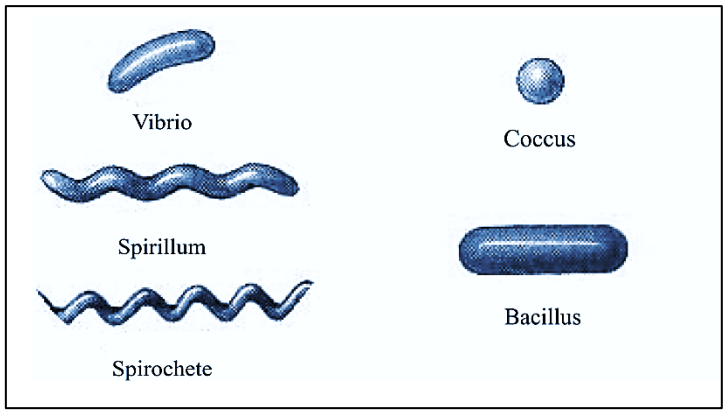


Figure 33. The fundamental bacterial shapes.

b) Other shapes

Although bacterial morphology is primarily classified based on three basic shapes, other bacterial shapes can exist:

- **Actinomycetes:** Branching filamentous bacteria. Example: *Streptomyces* species (Figure 34 a).
- **Mycoplasmas:** Cell wall-deficient bacteria with pleomorphic morphology (Figure 34 b).

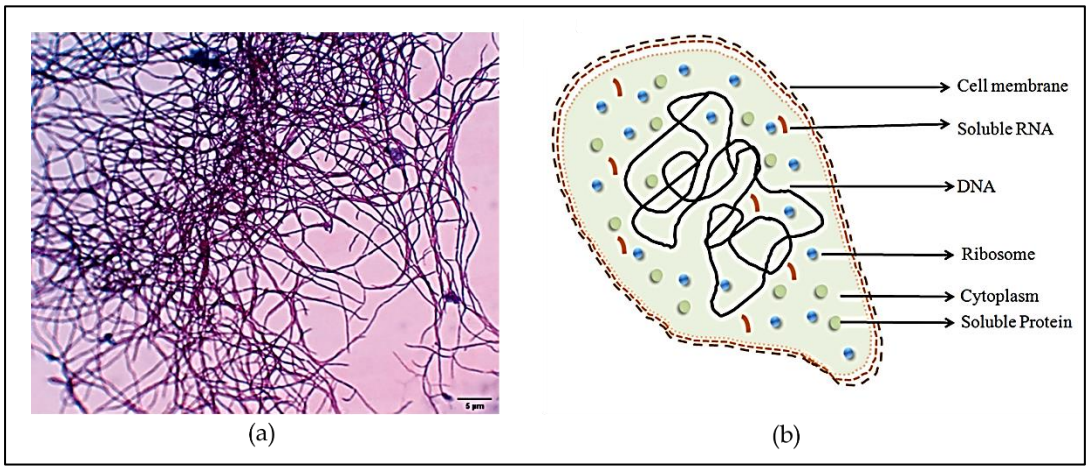


Figure 34. Other bacterial shapes. **(a):** The filamentous bacteria *Streptomyces* sp. (scale bar: 5 µm); **(b):** Representational image of *Mycoplasma* sp.

1.1.2. Arrangements of bacterial cells

Bacteria often form characteristic arrangements after cell division, which can be useful for identification:

a) Cocci arrangements

- **Monococci:** individual cocci.
- **Diplococci:** Pairs of cocci (e.g., *Neisseria*).
- **Streptococci:** Chains of cocci formed by cells dividing in one plane (e.g., *Streptococcus pyogenes*).
- **Tetrads:** Groups of four cocci dividing in two planes (e.g., *Tetragenococcus*).

- **Sarcina:** Cuboidal arrangements of eight cocci formed by regular division in three planes (e.g., *Sarcina ventriculi*).
- **Staphylococci:** Irregular grape-like clusters formed by irregular cell divisions in three planes (e.g., *S. aureus*). Figure (35) represents the different arrangements of cocci.

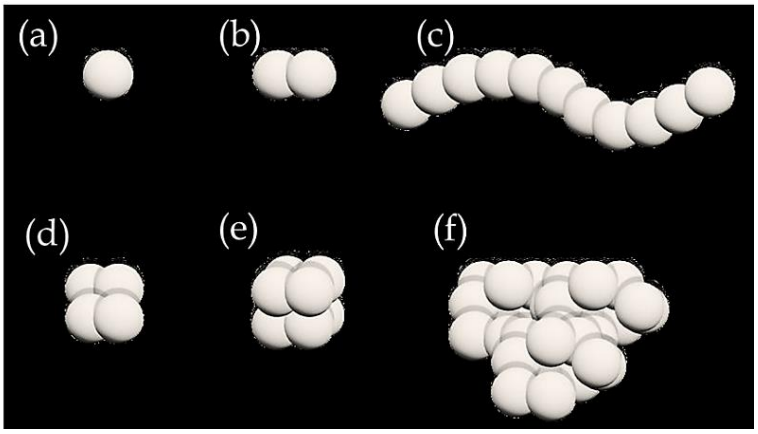


Figure 35. Graphical illustration of cocci arrangements. **(a):** Monococcus; **(b):** Diplococcus; **(c):** Streptococcus; **(d):** Tetacoccus; **(e):** Sarcina, and **(f):** Staphylococcus.

b) Bacilli arrangements

- **Single bacilli:** Present as individual cells (e.g., *Bacillus cereus*).
- **Diplobacilli:** Pairs of bacilli (e.g., *Moraxella bovis*).
- **Streptobacilli:** Bacilli are arranged in chains (e.g., *Streptobacillus moniliformis*).
- **Coccobacilli:** Resemble both cocci as well as bacilli. They are shorter in size and appear stumpy (e.g., *Haemophilus influenza*).
- **Palisades:** Side-by-side arrangement resembling a fence (e.g., *Corynebacterium diphtheria*). Figure (36) represents the different arrangements of bacilli.

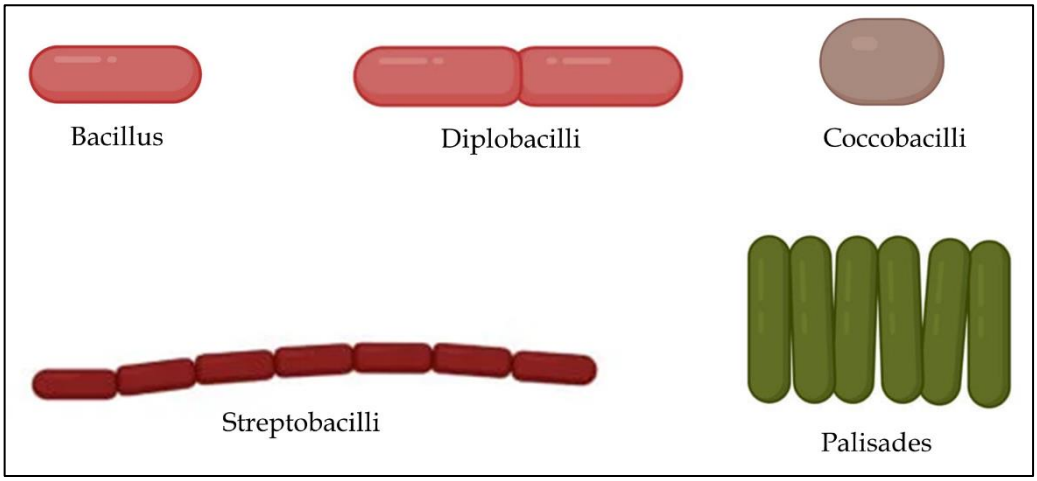


Figure 36. Arrangement of bacilli.

2. Bacterial Cell Structure

The bacterial cell consists of several structures, each with specific functions. Three categories exist for the organization of bacterial structure:

- **Internal structures:** Cytoplasm, nucleoid, bacterial chromosome, plasmid, ribosomes, endospores and storage granules.
- **Cell envelope:** cell membrane, cell wall.
- **External structure:** appendages and coverings, including flagella, fimbriae, sex pili, and capsule.

2.1. Cell envelope

2.1.1. Cell wall

The cell wall is a rigid structure that gives shape to the cell and prevents osmotic lysis. It is chemically composed of peptidoglycan (also called murein), which is a heterogeneous polymer comprising two distinct types of amino sugars: N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) linked together by β-(1,4) glycosidic bonds. The NAM is linked to a peptide chain of amino acids (2 alanine, glutamic acid, and lysine or diaminopimelic acid). These together form a monomer of peptidoglycan. The peptidoglycan unit (monomer) is linked to a second unit in an adjacent chain by a peptide cross-link, which forms a three-dimensional network and represents one layer of the wall (Figure 37).

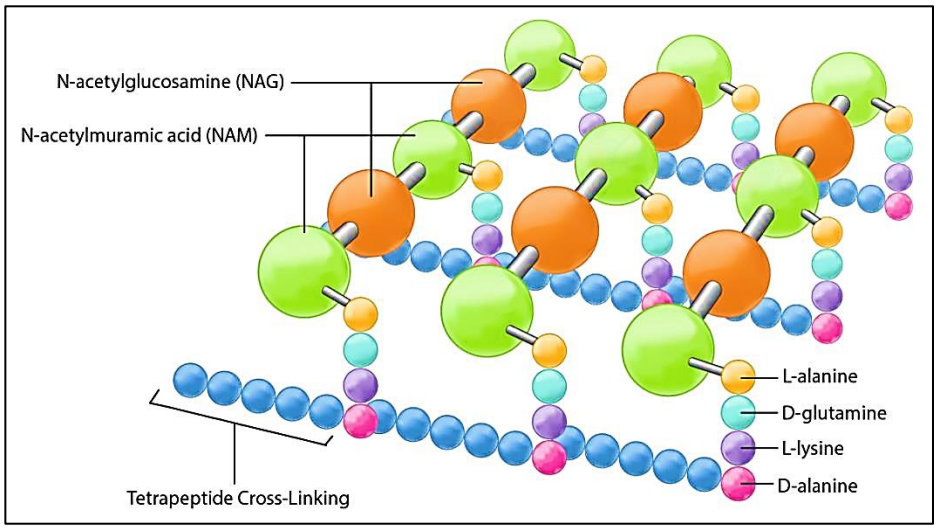


Figure 37. The structure of peptidoglycan (murein) in bacterial cell wall.

Based on cell wall structure, bacteria are classified into two major groups:

a) Gram-positive bacteria

The Gram-positive bacterial cell wall has different components that constitute its structure. It is composed of a thick homogenous peptidoglycan layer (about 80% of the cell wall). Lipoteichoic acids (anchored in the cytoplasmic membrane) and teichoic acids are present and are antigenic. Teichoic acids are covalently linked to the peptidoglycan in some Gram-positive bacteria. The outer

membrane, phospholipids and lipoproteins are absent. The cell wall retains crystal violet during the Gram staining procedure.

b) Gram-negative bacteria

The Gram-negative bacterial cell wall is more complex. It is mainly composed of a thin peptidoglycan layer (about 3-12% of the cell wall) in the periplasmic space and an outer membrane comprising lipopolysaccharide (LPS) and phospholipids. The most important outer membrane function is to serve as a protective barrier, preventing or slowing the entry of bile salts, antibiotics, and other toxic substances that might kill or injure the bacterium. The outer membrane is more permeable than the plasma membrane and permits the passage of small molecules like glucose and other monosaccharides due to the presence of special porin proteins.

Lipoproteins are present and cross-link the outer membrane and peptidoglycan layer. The LPS consists of lipid A (endotoxin), core polysaccharide, and O-antigen (O-polysaccharide). Teichoic acids are absent in the Gram-negative cells. The cell wall does not retain crystal violet during Gram staining. The Figure (38) illustrates the structure of both Gram-positive and Gram-negative bacterial cell walls.

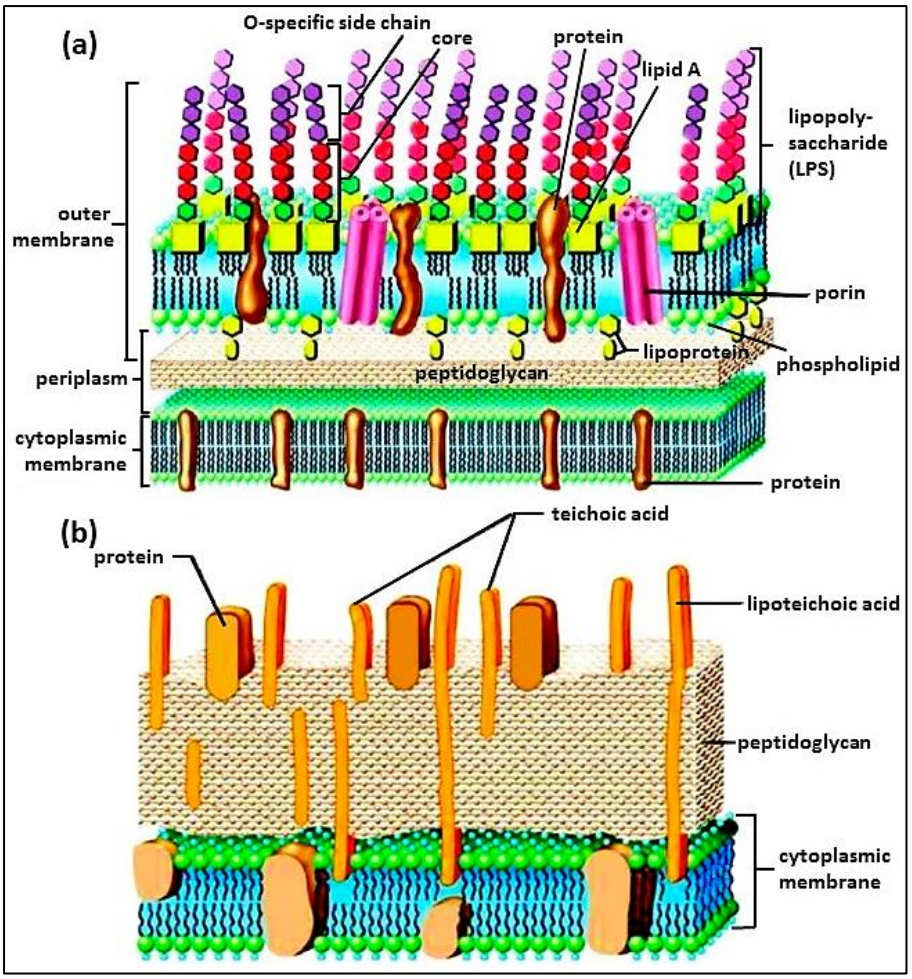


Figure 38. A schematic structure of bacterial cell wall. **(a):** Gram-negative bacteria; **(b):** Gram-positive bacteria.

2.2. Cytoplasmic membrane

The cytoplasmic membrane (plasma membrane) is present immediately beneath the cell wall. It is composed of a phospholipid bilayer and acts as a semi-permeable (selective) barrier. It also plays a crucial role in cellular processes, including energy generation, houses transport systems, and contains enzymes for various metabolic functions. Phospholipids have polar (hydrophilic) heads and non-polar (hydrophobic) tails, resulting in an amphipathic nature that enables membrane fluidity (Figure 39a). A pure phospholipid membrane only allows water, gasses, and a few small molecules to move freely through it.

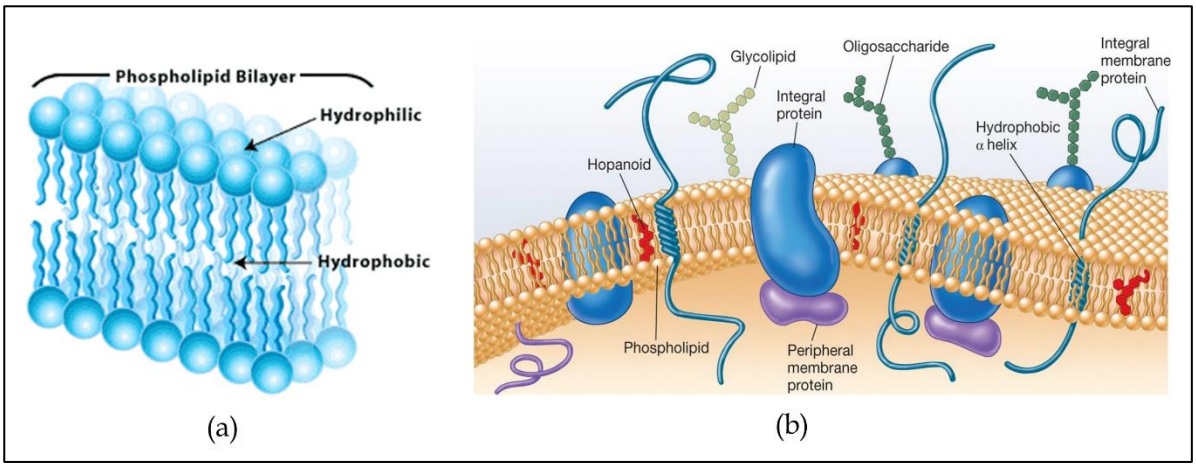


Figure 39. The cytoplasmic membrane of bacterial cell. **(a):** The phospholipid bilayer; **(b):** The structure of the cytoplasmic membrane.

The cytoplasmic membrane also contains peripheral membrane proteins (bound to one surface of the membrane) and integral membrane proteins (embedded in the membrane), which transport molecules across the membrane. Bacterial membranes notably lack sterols in their composition, but some incorporate hopanoids to enhance membrane rigidity and stability, unlike eukaryotic cell membranes (Figure 39b).

- **Transport across the cell membrane**

The cell membrane regulates the entry and exit of substances (nutrients, ions, waste products, etc.). It acts as a barrier that selectively allows molecules to pass through:

- **Passive transport:** Diffusion of small molecules (e.g., oxygen, water, and carbon dioxide) from high concentration to low concentration across the membrane. Movement of molecules is not energy-dependent.
- **Facilitated diffusion:** Similar to passive diffusion, but involves the use of carrier proteins to assist in the movement of larger or charged molecules (e.g., glucose and amino acids).
- **Active transport:** Energy-dependent processes (often via ATP or proton motive force) to pump substances against their concentration gradient. It involves carrier proteins (pumps or transporters).

2.2. Internal structures

2.2.1. Cytoplasm

Bacterial cytoplasm is a colloidal system composed of water (80%), proteins, salts, and other solutes (20%). It also contains various structures, including ribosomes (70S), storage inclusions, nucleoid and plasmids.

2.2.2. Ribosomes

Ribosomes are complex protein/ARN structures. They are the sites of protein synthesis and distributes in a large number in the cytoplasm. Their number varies from 10 000 to 15 000 in a cell. Bacterial ribosomes consist of two subunits: 50S large subunit (5S rRNA, 23S rRNA and 34 proteins), and 30S small subunit (16S rRNA and 21 proteins). During the protein synthesis, the two subunits combine to form a functional 70S ribosome.

2.2.3. Inclusions

a) Storage inclusions

Storage inclusions are granules of organic or inorganic polymers that are stored by the cell when there is an excess of nutrients in the environment. They serve as a storage reserve of nutrients, metabolic end products, carbon, energy, and building blocks.

The most important storage granules in bacterial cells include polysaccharide granules (e.g., glycogen granules), sulfur granules, lipid granules containing polymer of poly-β-hydroxybutyric acid (PHB), and volutin granules (also known as metachromatic granules) containing inorganic polyphosphate and function as energy and phosphate reserves for cell metabolism (Figure 40).

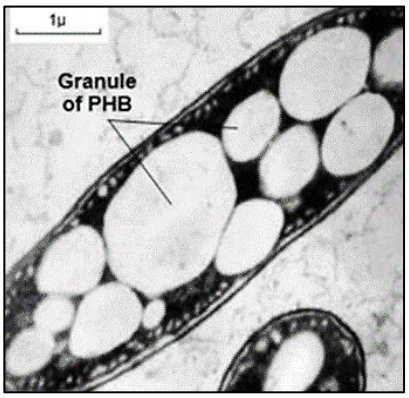


Figure 40. Lipid granules within bacterial cells include poly-β-hydroxybutyric acid (PHB).

b) Other inclusions

- **Gas vacuoles:** Some bacterial species, particularly those found in aquatic environments (e.g., halophilic bacteria and photosynthetic bacteria), contain gas vacuoles, which are small protein-bound structures that contain gas. These vacuoles help regulate the buoyancy of bacterial cells, maintaining an optimal depth for growth. By changing their buoyancy, these vacuoles enable the bacteria to float towards the surface or sink to deeper layers of water (Figure 41a).

- **Magnetosomes:** Found in aquatic bacteria. They contain magnetite particles in chains (known as magnetosomes) for orientation in Earth's magnetic field (Figure 41b).

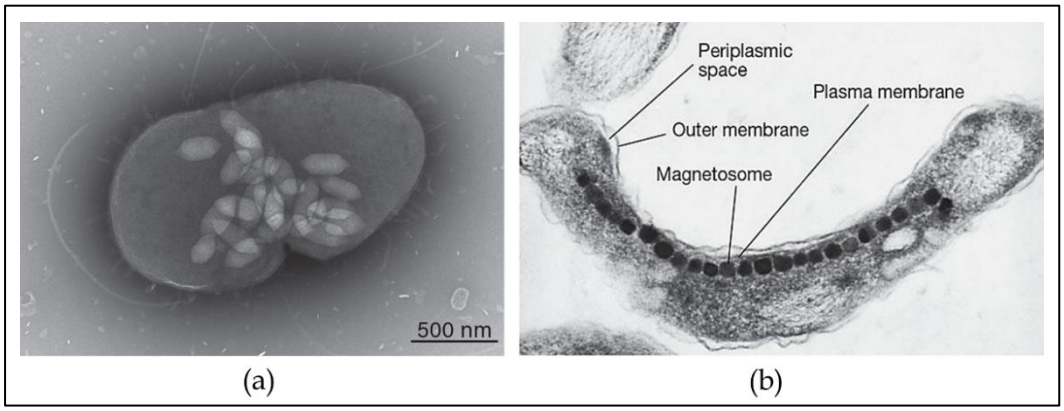


Figure 41. Other bacterial cell inclusions. **(a):** Gas vacuoles (scale bar: 500 nm); **(b):** Magnetosomes.

2.2.4. Photosynthetic pigments

The cytoplasm of certain photosynthetic bacteria, such as purple and green sulfur bacteria, contains membrane structures designated as chromatophores. These structures are invaginations or extensions of the plasma membrane containing bacterial chlorophyll pigments and other accessory pigments (e.g., carotenoids) that facilitate the conversion of solar energy into chemical energy.

In cyanobacteria, however, the photosynthetic pigments are not located in chromatophores but in thylakoids, which are distinct from chromatophores. Thylakoids contain chlorophyll and other metabolite pigments (phycobiliproteins) for light absorption (photosynthesis).

2.2.5. The genetic material

a) Nucleoid

In bacteria, the nucleoid (sometimes referred to as primitive nucleus) consists of chromatin material that is difficult to observe without staining with special dyes. It is devoid of nuclear membrane and nucleolus. The nucleoid contains the bacterial genome, which consists of a single, circular, double-stranded DNA molecule. Bacterial DNA is devoid of histones and referred to as bacterial chromosome (Figure 42).

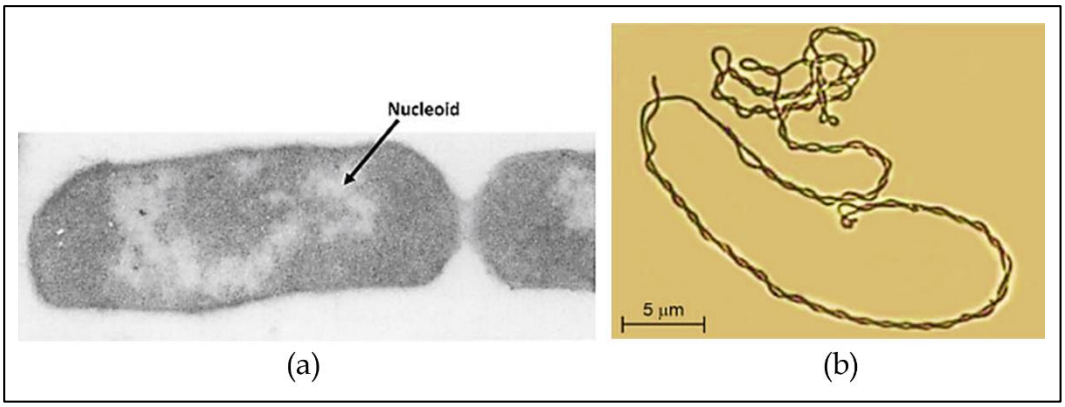


Figure 42. Bacterial genetic material. **(a):** Nucleoid; **(b):** Bacterial DNA; **(c):** Bacterial plasmid.

b) Plasmid

Plasmids are circular extra-chromosomal DNA molecules that replicate independently and carry non-essential genes (Figure 43). They can be transferred between bacteria.

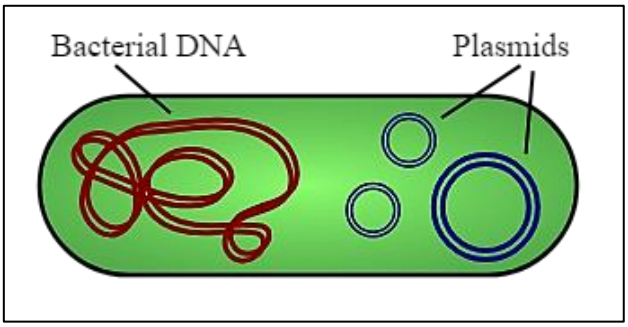


Figure 43. Bacterial plasmids.

The presence of plasmids confers different advantages to the bacterial cells, including antibiotic resistance, heavy metal resistance, virulence, environmental adaptability and persistence, utilization of different nutrients, and biodegradation of a variety of toxic substances (e.g., toluene and other organic hydrocarbons, herbicides, and pesticides).

Based on bacterial host properties, plasmids can be classified into different types, including:

- **Conjugative plasmids:** Also known as F (fertility) plasmids, contain genes that enable the transfer of plasmids between bacteria via conjugation.
- **Resistance plasmids (R plasmids):** Carry genes for resistance to antibiotics or other toxic substances.
- **Colicinogenic plasmids (Col plasmids):** Carry genes that produce colicins, which are toxic proteins that can kill other closely related bacterial species.
- **Virulence plasmids:** Contain genes that contribute to the pathogenicity of bacteria, making them more virulent.
- **Degradative plasmids:** Carry genes that enable bacteria to metabolize unusual substances, such as hydrocarbons, pesticides, or other environmental pollutants, allowing them to survive in diverse environments.

2.2.7. Endospores

Bacterial endospores are highly resistant, dormant structures formed by some bacteria species (e.g., *Bacillus*, *Clostridium*) under extreme environmental conditions that are unfavorable for vegetative cells (e.g., heat, desiccation, radiation, and chemicals). Bacterial endospores appear in different positions within the cells, including central, subterminal, terminal, and swollen sporangium (Figure 44).

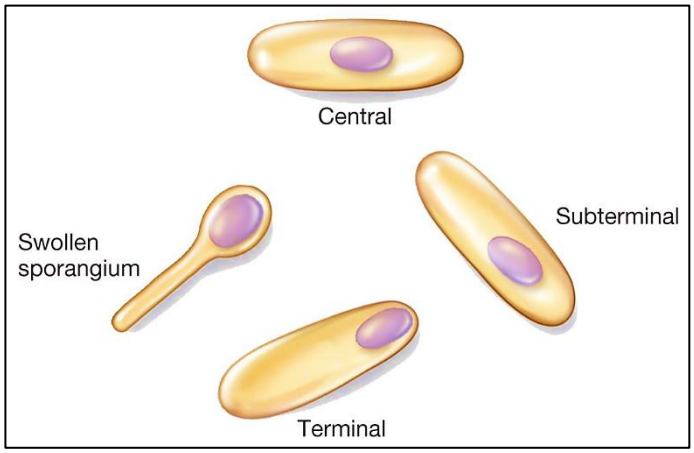


Figure 44. Bacterial endospores positions.

An endospore is a complex structure composed of several distinct layers that contribute to its resistance and survival. The major structural components include the exosporium (surrounding the spore coat), spore coat (protecting the inner core), outer membrane, cortex (containing peptidoglycan and playing a role in heat resistance), germ cell wall, inner membrane (maintaining the integrity of the endospore), and core (containing bacterial DNA, ribosomes, and essential enzymes necessary for the germination and reactivation of the spore) (Figure 45).

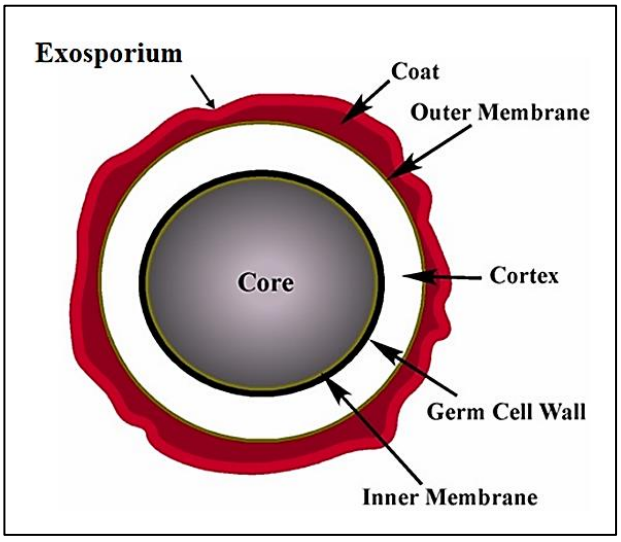


Figure 45. Structure of bacterial endospore.

Endospores are produced via a process called sporulation (Figure 46). They can remain dormant for long periods until conditions improve, and they germinate into active, vegetative bacterial cells.

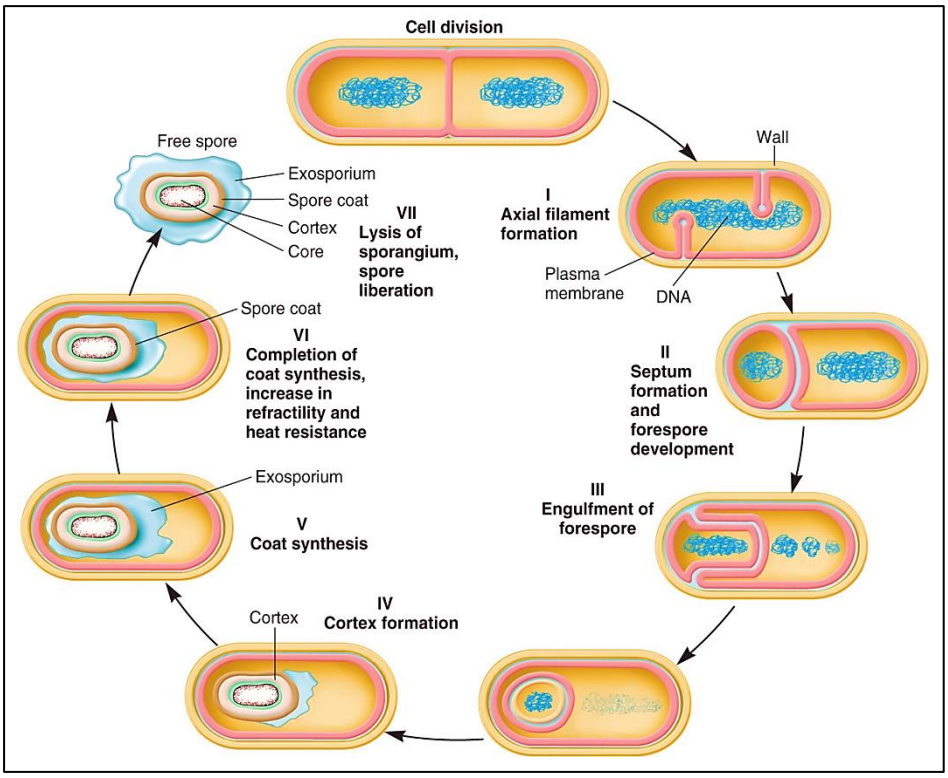


Figure 46. The sporulation process: bacterial endospore formation.

2.3. External structures

2.3.1. Capsule and slime layer

The capsule/slime layers are the outermost layer of the bacteria (extracellular). They are condensed, well-defined layers surrounding the cell wall. Capsules are usually composed of polysaccharides (e.g., *S. pneumoniae*) and occasionally polypeptide chains of amino acids (e.g., *B. anthracis*). They are secreted by the cell into the external environment. Capsules are relatively impermeable structures. They provide protective advantages to bacteria, including protection against phagocytosis, prevention of desiccation, and contribution to virulence.

Slime layers facilitate adherence to surfaces and contribute to biofilm formation. Slime layers can also be used as a food reserve for the cell.

2.3.2. Flagella

Flagella (singular: flagellum) are long, helical filaments extending from the cell membrane through the cell wall to the exterior of the bacterial cell. Flagella are organs of locomotion and their loss impacts bacterial motility but does not affect viability. Flagella are characteristic of many bacterial species but are not universally present in all bacteria. Their number, position, and arrangement vary with species.

Flagella are complex structures (Figure 47) that consist of:

a) **Filament:** Composed of flagellin proteins assembling into a helical structure, and giving the filament its rigidity and ability to rotate effectively. The filament contains an open channel that

extends along its length. Flagellin subunits, synthesized within the bacterial cell, are transported through this central channel to the tip of the filament.

b) **Hook:** A curved, tubular structure that acts as a flexible joint connecting the filament and the basal body. It is composed of FlgE proteins assembling into a helical structure, providing both flexibility and strength.

c) **Basal body:** A complex structure functions as a rotary motor that powers the movement of the flagellum. It is composed of:

- **Cytoplasmic ring (C Ring):** Located on the cytoplasmic side and is responsible for switching the direction of rotation and is crucial for the motor's function.
- **MS ring:** Embedded in the cytoplasmic membrane, providing structural support and acting as a foundation for other flagellar components.
- **P and L rings:** Specific to Gram-negative bacteria. The P ring is located in the peptidoglycan layer, while the L ring is situated in the outer membrane providing additional support.

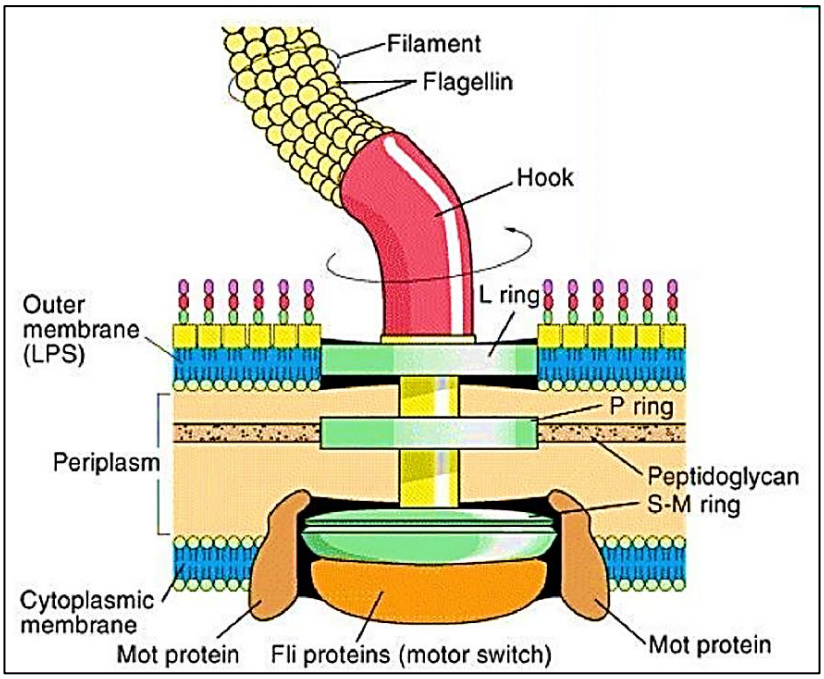


Figure 47. Structure of flagellum in Gram-negative bacteria.

Based on flagellar arrangement, bacteria are classified as (Figure 48):

a) **Atrichous:** lack flagella entirely and are non-motile (e.g., *Lactobacillus*).

b) **Monotrichous:** Single flagellum at one end (e.g., *Vibrio cholerae*).

c) **Lophotrichous:** Tuft of flagella at one end (e.g., *Spirillum serpens*).

d) **Amphitrichous:** Flagella at both ends (e.g., *Nitrosomonas*).

e) **Peritrichous:** Flagella distributed around the cell (e.g., *E. coli*).

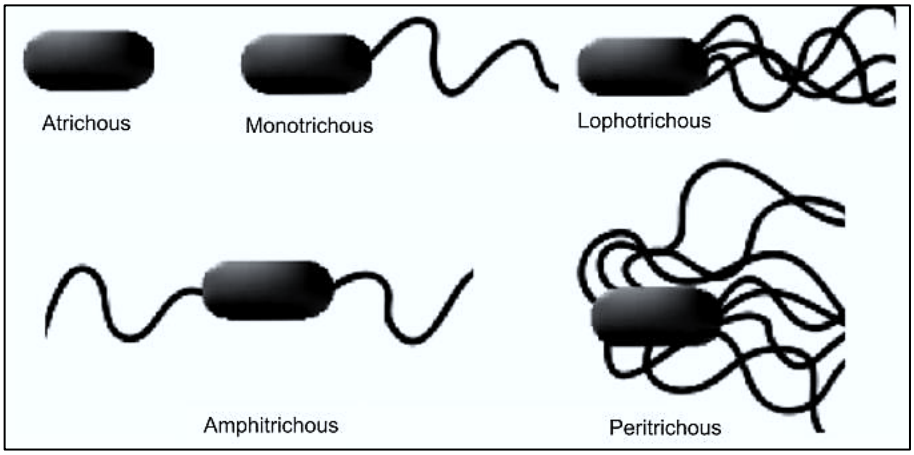


Figure 48. Different flagellar arrangements in bacteria.

- **Bacterial motility and taxis**

  Bacteria can move in response to various external stimuli through different types of taxis:

  - **Chemotaxis:** Movement toward chemical attractant (e.g., nutrients) or away from harmful substances. Bacteria sense chemical gradients using chemoreceptors and adjust their movement accordingly (e.g., *E. coli*).
  - **Phototaxis:** Movement toward or away from light sources (e.g., Cyanobacteria).
  - **Aerotaxis:** Movement in response to oxygen concentration. Bacteria move toward optimal oxygen levels for their metabolism.
  - **Magnetotaxis:** Movement along magnetic field lines. Magnetosomes present in magnetotactic bacteria (e.g., *Magnetospirillum*) act like a compass needle, aligning the bacteria with Earth's magnetic field.

2.3.3. Pili and Fimbriae

They are proteinaceous hair-like appendages that extend from the surface of many bacterial cells. There are two types of pili: common pili (fimbriae) and sex pili. Common pili, like fimbriae (singular: fimbria), are generally thin, short in length, numerous, involved in adhesion to surfaces (Figure 49). Fimbriae usually function to facilitate the attachment of a bacterium to a surface (e.g. to form a biofilm) or to other cells (e.g. animal cells during pathogenesis). Fimbriae are antigenic structures.

b) Sex pili

Sex pili (singular pilus) are longer, thicker, and present in low numbers (Figure 49). They are involved in bacterial conjugation, a process in which genetic material is transferred between bacteria. The genes encoding for sex pili formation are located on F plasmids.

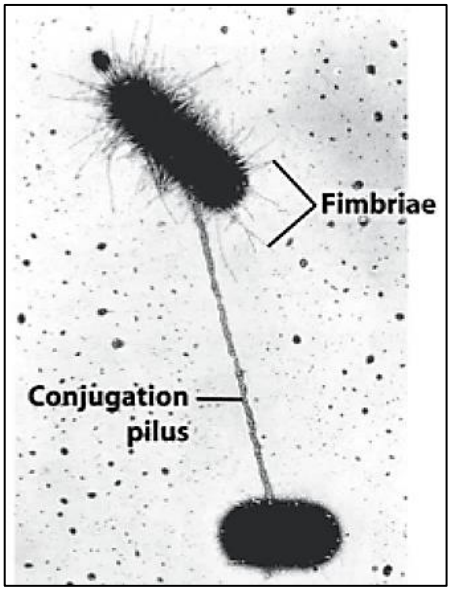


Figure 49. Sex pili and fimbriae in bacteria.

Chapter III

Bacterial Nutrition and Metabolism

Chapter III. Bacterial Nutrition and Metabolism

1. Bacterial Nutrition

1.1. Nutritional requirements

1.1.1. Definition of nutrients

Nutrients are essential chemical substances required by bacteria for several vital processes, including energy production, metabolic functions, cellular growth, and reproduction. These substances are essential for cellular processes and must fulfil the following roles:

- ✓ Act as key constituents of cellular structures.
- ✓ Support the function of enzymes and transport systems.
- ✓ Provide the necessary components for generating biologically usable energy.

The cellular content of a typical bacterial cell can be divided into two main components:

- **Water:** Constitutes approximately 80–90% of the cell's total weight.
- **Dry Matter:** Represents about 10% of the cell's weight, and it is composed of proteins (52%), polysaccharides (17%), lipids (9%), RNA (16%), and DNA (3%).

1.1.2. Types of nutrients

Nutrients are classified into distinct categories based on their function and their role in bacterial life processes. These categories include macronutrients, micronutrients, and growth factors. Generally, about 95% of the dry weight is composed of carbon, oxygen, hydrogen, nitrogen, and phosphorus. The remaining 5% is comprised of various trace elements or micronutrients.

a) Macronutrients

Macronutrients are nutrients that are required by bacteria in large quantities and are essential for their growth, metabolism, and cellular structure. They include carbon (C), nitrogen (N), oxygen (O), hydrogen (H), phosphorus (P), and sulfur (S).

- **Carbon (C):** The most abundant element in bacterial cells (approximately 50% of bacterial dry weight). It is indispensable for synthesizing cellular materials, including carbohydrates, lipids, proteins and nucleic acids. It serves as a major energy source for bacterial growth and metabolism.

Bacteria obtain carbon from various sources, depending on their metabolic capabilities. Based on these sources, bacteria can be classified as:

- ➢ **Autotrophs:** Use inorganic carbon sources, such as carbon dioxide (CO_2), as their primary carbon source through processes like photosynthesis or chemosynthesis.
- ➢ **Heterotrophs:** Can only utilize carbon from organic sources, such as carbohydrates, amino acids, fatty acids, and alcohols. They are widely distributed and most abundant in nature.

- **Nitrogen (N):** Nitrogen constitutes approximately 14% of the dry cell weight of bacteria. It is essential for the synthesis of amino acids (building blocks of proteins), nucleotides (for DNA and RNA), and other nitrogen-containing compounds. Nitrogen can be obtained from various sources, including inorganic forms (e.g., ammonium and nitrate, commonly utilized by many bacteria) and organic forms (e.g., amino acids and peptides). Certain bacteria (nitrogen-fixing bacteria) can utilize atmospheric nitrogen (N_2).
- **Phosphorus (P):** A key component of nucleic acids (DNA and RNA), ATP (the energy currency of cells), and phospholipids in cell membranes. Phosphorus is usually absorbed by bacteria from their environment as phosphate ions (PO_4^{3-}).
- **Sulfur (S):** Necessary for synthesizing certain amino acids (e.g., cysteine and methionine), growth factors (e.g., coenzyme A and biotin). Bacteria can utilize inorganic sulfur in the form of sulfate (SO_4^{2-}) or organic sulfur compounds, such as sulfur-containing amino acids (cysteine and methionine) and vitamins (biotin, niacin), depending on their metabolic capabilities.
- **Oxygen (O) and Hydrogen (H):** Oxygen is required for aerobic respiration in many bacteria, serving as the final electron acceptor in the electron transport chain. However, some bacteria are anaerobic and do not require oxygen for growth.

  Hydrogen is involved in maintaining cellular pH balance and is a component of water, energy transfer in molecules (e.g., NADH), and a constituent of all organic molecules (carbohydrates, lipids, proteins, and nucleic acids).
- **Magnesium (Mg^{2+}):** Essential for stabilizing nucleic acids, ribosomes, membranes, and enzyme activation (a cofactor for many enzymes, including ATPases). It is also a fundamental component of bacterial chlorophyll pigments.
- **Potassium (K^+):** Critical for enzyme activation (a cofactor for many enzymes), pH regulation (maintaining intracellular pH), osmotic balance and cell equilibrium.
- **Calcium (Ca^{2+}):** Important for cell wall stability, enzyme activation, and regulation of metabolic processes. It is also involved in sporulation (component of endospores), spore heat resistance, and biofilm formation.
- **Iron (Fe):** Essential cofactor for many enzymes. It participates in fundamental metabolic pathways, including electron transport (component of cytochrome), cellular respiration, and DNA synthesis.

b) Micronutrients and trace elements

Micronutrients, also known as trace elements, are required in small amounts (trace) but play a variety of crucial roles in bacterial physiology. Bacteria need trace elements for their survival, growth, enzyme activation, and essential metabolic functions.

- **Sodium (Na^+):** Required for cell equilibrium.

- **Zinc (Zn):** Essential for cell growth and participates in regulating various enzymatic reactions. In bacterial cells, more than 3% of proteins and enzymes are zinc-containing proteins, which can be used as intrinsic metalloenzymes and cofactors for enzymes, such as those essential for DNA replication (bacterial DNA polymerase) and protein synthesis (essential for the function of ribosomal enzymes).
- **Manganese (Mn):** A cofactor for multiple enzymatic systems. It protects bacteria against oxidative stress by acting as a cofactor for superoxide dismutase, which neutralizes harmful superoxide radicals. Manganese is also integral to various metabolic pathways, including amino acid biosynthesis, carbohydrate metabolism, and peptide synthesis.
- **Copper (Cu):** A vital element for bacteria. It plays a role in various processes, including respiration, iron acquisition, oxygen scavenging, signal transduction, and defense against oxidative stress. The significance of copper manifests in its active participation in cytochrome oxidases during aerobic respiration and ATP synthesis.
- **Molybdenum (Mo):** A key component of molybdoenzymes (cofactor of a series of oxidoreductases), which are involved in nitrogen fixation.
- **Cobalt (Co):** Required by some bacteria for the biosynthesis of vitamin B12 (cobalamin).
- **Selenium (Se):** Functions as a component of selenoproteins (modified amino acids) that catalyze redox reactions (oxidoreductase enzymes). It also functions as an antioxidant, protecting bacterial cells against oxidative stress.
- **Nickel (Ni):** Required for urease, hydrogenase, and some CO dehydrogenases.

c) Growth factors

Growth factors are organic compounds that certain bacteria (termed fastidious bacteria) are unable to synthesize these substances independently and must therefore obtain them from their environment. These factors are required in small quantities in the medium to support the optimal growth and reproduction of bacteria. They are essential for a wide range of cellular processes, including enzyme function, nucleic acid synthesis, and overall metabolic processes. The specific requirements for growth factors vary among bacterial species, with some requiring multiple factors while others need only specific ones for optimal growth. Growth factors primarily include:

- ✓ Vitamins, including thiamine (B_1), essential for carbohydrate metabolism; riboflavin (B_2), a component of FAD and FMN; niacin (B_3), a component of NAD and NADP; pantothenic acid (B_5), essential for coenzyme A synthesis; biotin (B_7), required for carboxylation reactions; folic acid (B_9), required for one-carbon metabolism; cobalamin (B_{12}), required for methyl transfer reaction; and vitamin K, required by some anaerobic bacteria.
- ✓ Amino acids (e.g., arginine and tryptophan).
- ✓ Nucleic acid bases (purines and pyrimidines).

1.2. Energy requirements of bacteria

Bacteria require energy to perform all cellular processes, including metabolism, cell division, and maintenance of cell structures. Without energy, bacteria would not be able to carry out critical functions such as protein synthesis, replication, and transport of nutrients.

1.2.1. Sources of energy for Bacteria

In order to grow, bacteria need a source of energy. The energy comes from the oxidation of organic or inorganic compounds. In bacteria, two principal sources of energy may be used.

a) Phototrophs

Phototrophs are a type of bacteria that can use solar energy in their metabolic processes. They contain photosynthetic pigments (like chlorophyll in plants) and can convert light into chemical energy in the cell through photosynthesis. Example: Cyanobacteria and purple sulfur bacteria.

b) Chemotrophs

Chemotrophs use chemical compounds (organic or inorganic chemicals) as energy sources. They can be divided into two categories based on the types of chemicals they use to produce energy:

- **Chemoorganotrophs:** They obtain their energy from organic compounds (such as sugars, amino acids, and fatty acids). For instance, *E. coli* uses glucose to produce energy through aerobic or anaerobic respiration.
- **Chemolithotrophs:** They obtain their energy from inorganic compounds (e.g., hydrogen gas, sulfur, or iron). For instance, sulfur bacteria (e.g., *Thiobacillus*) use hydrogen sulfide (H_2S) as an electron donor to produce energy.

1.3. Nutritional types

Based on their nutrition types, bacteria can be classified into four groups: photoautotrophs, photoheterotrophs, chemoautotrophs, and chemoheterotrophs.

1.3.1. Photoautotrophs

They are referred to as photosynthetic bacteria. They use inorganic carbon (CO_2) as their main source of carbon. These bacteria can capture light energy by photosynthetic pigments (mainly chlorophyll) and use this energy to drive the conversion of CO_2 into organic materials (photosynthesis). Examples include cyanobacteria (oxygenic phototrophs), green sulfur bacteria, purple sulfur bacteria, and green non-sulfur bacteria.

1.3.2. Photoheterotrophs

Photoheterotrophic bacteria are a group of microorganisms that utilize sunlight as an energy source and do not require carbon fixation. They use organic substances (e.g., sugars, fatty acids, or other organic molecules) for their carbon needs instead of using inorganic CO_2. The best-known of photoheterotrophic bacteria are purple non-sulfur bacteria, often found in aquatic environments with organic carbon.

1.3.3. Chemoautotrophs

They are a group of bacteria that obtain energy by oxidizing inorganic substrates in their environment, and at the same time, they utilize and fix CO_2 and convert it into cell material (organic substances) in the Calvin-Benson cycle. The energy produced is used to fix CO_2, and the organic compounds derived from these fixation pathways are regarded as the primary carbon source that sustains the cells.

Examples of chemoautotrophic bacteria include sulfur-oxidizing bacteria, such as the genus *Thiobacillus*, which oxidizes sulfur compounds like hydrogen sulfide (H_2S), thiosulfate, or elemental sulfur to sulfate. In the nitrogen cycle, the genus *Nitrosomonas* (nitrifying bacteria) oxidizes ammonia (NH_3) to nitrite (NO_2^-), while *Nitrobacter* (Nitrifying bacteria) further oxidizes NO_2^- to nitrate (NO_3^-). Additionally, the species *Acidithiobacillus ferrooxidans* (an iron-oxidizing bacteria) derives energy by oxidizing ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) and sulfur compounds. These bacteria play essential roles in biogeochemical cycles and environmental processes.

1.3.4. Chemoheterotrophs

Chemoheterotrophs are a group of bacteria that oxidize organic compounds (e.g., carbohydrates, lipids, proteins, amino acids, and nucleic acids) to obtain their energy. They use these organic compounds as their primary source of carbon. Chemoheterotrophs employ both respiration and fermentation as a means of harvesting energy.

Most bacteria are chemoheterotrophic. They can be further classified into three main categories:

a) Saprophytic bacteria

Saprophytic bacteria grow in dead and organic decaying matter (e.g., leaves, fruits, vegetables, meat, animal feces, leather, humus, etc.). These bacteria secrete enzymes to digest the food and absorb it. The enzymes are secreted to break down complex compounds such as carbohydrates and protein into simpler soluble compounds, which are easily absorbed. Examples include *Bacillus mycoides*, *B. ramosus*, *Acetobacter* etc.

b) Symbiotic bacteria

Symbiotic bacteria live in close association with other organisms as symbionts so that they both benefit each other, and neither of them is harmed. A prevalent example is *Rhizobium* spp. These bacteria inhabit the roots of leguminous plants. They convert atmospheric nitrogen into nitrogenous compounds, which are utilized by plants. The plant provides nutrients and protection to the bacteria in exchange.

c) Parasitic bacteria

Parasitic bacteria live in or on a host organism and derive nutrients at the host's expense. They establish a relationship where the bacteria benefit while the host may be harmed. Parasitic bacteria that cause diseases in their hosts are known as pathogenic bacteria. Examples include *B. anthracis* (anthrax), *Clostridium tetani* (tetanus), *M. tuberculosis* (tuberculosis), and *V. cholerae* (cholera). Obligate

intracellular parasitic bacteria, such as *Rickettsia* and *Chlamydia* species, cannot reproduce outside host cells and are also pathogenic.

Not all parasitic bacteria are pathogenic. Some establish commensal relationships where they benefit without significantly affecting the host. Others may even form mutualistic relationships where both the bacteria and host benefit, such as certain gut bacteria that aid in digestion while receiving a protected environment and nutrients.

2. Bacterial Metabolism

2.1. Bacterial enzymes

2.1.1. Definition of enzymes

Enzymes are protein-based catalysts that accelerate the rate of cellular reactions under mild physiological conditions without being changed themselves.

2.1.2. General properties of enzymes

Bacterial enzymes possess different properties, including:

a) Catalytic function

Enzymes act as biological catalysts, significantly increasing the rate of metabolic reactions by lowering the activation energy required for these processes. This ensures that essential biochemical reactions occur efficiently under physiological conditions.

b) Specificity

Enzymes are highly specific to their substrates, meaning each enzyme can only catalyze specific reactions or act on particular substrates (e.g., urease hydrolyzes urea exclusively). This property is crucial for maintaining the organized and regulated flow of metabolic pathways.

c) Regulation

Enzyme activity in bacteria is tightly regulated to adapt to environmental changes and metabolic demands. Regulation occurs through mechanisms, such as feedback inhibition, allosteric modulation, and transcriptional control of enzyme synthesis.

d) Cofactor dependency

Many bacterial enzymes require cofactors (e.g., inorganic ions such as Mg^{2+}, Zn^{2+}, Fe^{3+})or coenzymes (e.g., NAD^+, FAD, and coenzyme A) to function properly. The cofactors assist in catalytic activity or stabilize enzyme-substrate interactions, while the coenzymes function as transient carriers of electrons or functional groups (e.g., acyl transfer by coenzyme A).

e) Environmental adaptability

Bacterial enzymes are highly adaptable, allowing bacteria to thrive in diverse environments. For example, some enzymes are stable and active under extreme conditions (e.g., high temperatures or acidic pH).

f) Diversity of functions

Bacteria produce a wide range of enzymes that participate in various metabolic processes, including carbohydrate metabolism (e.g., trehalase for trehalose degradation), nitrogen cycling (e.g., nitrifying enzymes), sulfur oxidation, and iron metabolism.

g) Role in stress response

Certain bacterial enzymes help protect against environmental stressors (e.g., oxidative stress). For instance, cytochrome oxidases protect bacteria from toxic small molecules like hydrogen peroxide and nitric oxide by facilitating efficient respiration even under low oxygen conditions.

h) Applications

Bacterial enzymes have practical applications in biotechnology and industry. For example, trehalose-degrading enzymes (e.g., trehalase) are utilized for carbohydrate processing, while sulfur-oxidizing enzymes are used in bioremediation.

2.1.3. Structure of enzymes

a) Types of enzymes

The structural organization of enzymes is essential for their function. Enzymes can exist in two forms:

- **Simple proteins:** composed only of protein component.
- **Complex molecules (Holoenzymes):** Consist of two parts, a protein component (apoenzyme) and a non-protein (cofactor) essential for catalytic activity. The apoenzyme is inactive in the absence of its non-protein cofactor. However, the holoenzyme is active when the cofactor is present (Figure 50).

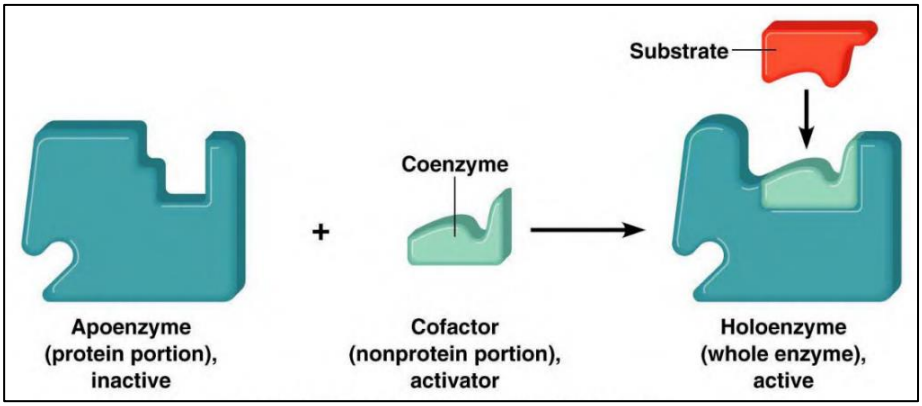


Figure 50. A schematic presentation of the inactive protein component (Apoenzyme) and the active holoenzyme (apoenzyme + cofactor).

b) Active site

The active site is a specific region within the enzyme, formed by specific amino acid residues, where substrate binding with high specificity and catalysis (transformation into products) occur (Figure 51).

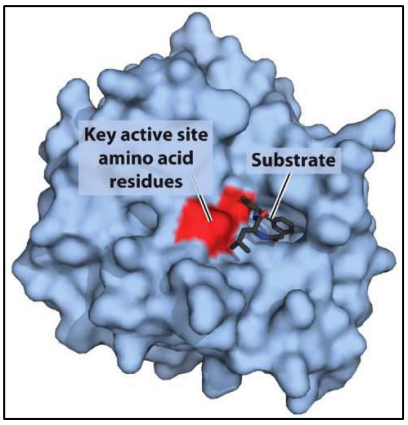


Figure 51. The active site.

The active site has two key regions:

- **Binding site:** Holds the substrate in place through temporary interactions such as hydrogen bonds, van der Waals forces, hydrophobic interactions, or electrostatic forces.
- **Catalytic site:** Facilitates the chemical transformation of the substrate by lowering the activation energy required for the reaction.

In addition to their active sites, some enzymes contain tightly bound prosthetic groups (e.g., heme in cytochromes) or loosely associated coenzymes that participate directly in catalytic activity. These structural features allow enzymes to perform highly specific biochemical transformations critical for bacterial metabolism.

c) Models of substrate binding

Two models describe substrate binding (Figure 52):

- **Lock-and-key model:** Where the active site is rigid and complementary to the substrate's shape.
- **Induced-fit model:** This proposes that the active site undergoes conformational changes upon substrate binding to achieve optimal interaction. The latter model better explains the flexibility observed in many bacterial enzymes.

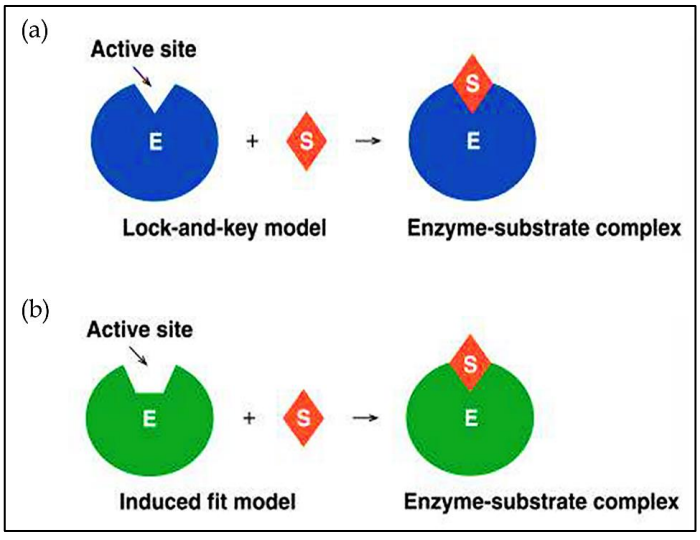


Figure 52. Models of substrate binding. **(a):** The lock-and-key model; **(b):** The induced fit model.

2.1.4. Classification of enzymes

Enzymes can be classified based on the type of chemical reaction catalyzed. This system divides enzymes into six major classes:

a) Oxidoreductases: Catalyze oxidation-reduction reactions, in which oxygen or hydrogen are gained (e.g., cytochrome oxidase, lactate dehydrogenase).

b) Transferases: Transfer functional groups such as amino or phosphate groups between donor and acceptor molecules (e.g., aminotransferases involved in nitrogen metabolism).

c) Hydrolases: Hydrolysis, addition of water (e.g., β-lactamase breaking down antibiotics like penicillin).

d) Lyases: Removal of groups of atoms without hydrolysis (e.g., fumarase in the Krebs cycle).

e) Isomerases: Facilitate molecular rearrangements within a molecule (e.g., alanine racemase in peptidoglycan synthesis).

f) Ligases: Join two molecules using energy usually derived from the breakdown of ATP (e.g., DNA ligase facilitating DNA repair).

3.1.5. Factors affecting enzyme activity

Several environmental and biochemical factors influence bacterial enzyme activity (Figure 53):

a) Temperature: Each enzyme has an optimal temperature at which it functions most efficiently. For mesophilic bacteria like *Escherichia coli*, this range is typically 30–40°C. Temperatures beyond this range can lead to denaturation or loss of enzymatic activity due to structural instability.

b) pH: The activity of bacterial enzymes is also pH-dependent. Cytoplasmic enzymes generally function near neutral pH (6–8), whereas periplasmic enzymes in certain pathogens may operate effectively under acidic conditions.

c) Substrate Concentration: Increasing substrate concentration enhances reaction rates until all active sites are saturated, at which point the reaction reaches a plateau (Vmax). This relationship is described by Michaelis-Menten kinetics.

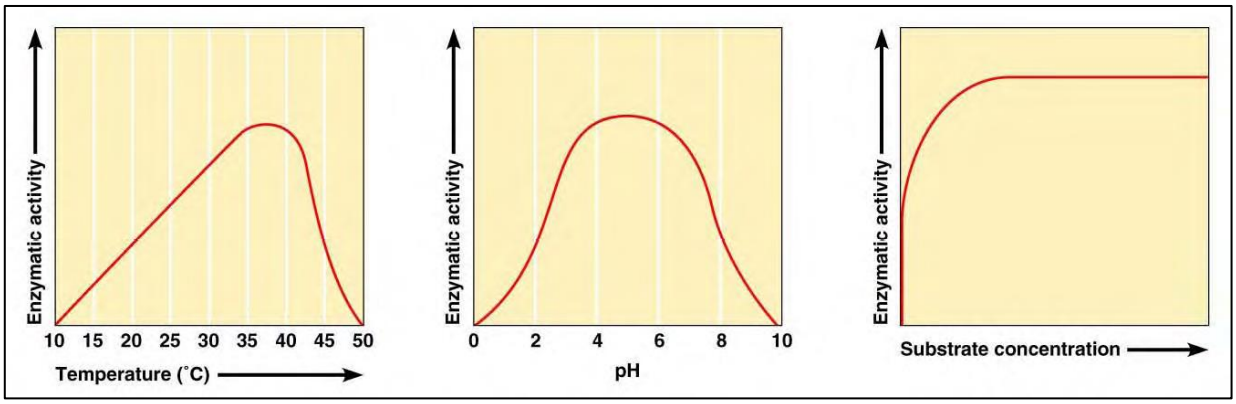


Figure 53. Factors that affect enzymes activity.

d) Inhibitors: Enzyme inhibitors can either compete with substrates for active sites (competitive inhibition) or bind allosterically to alter enzyme conformation (non-competitive inhibition). For instance, sulfa drugs inhibit bacterial dihydropteroate synthase by mimicking its natural substrate (Figure 54).

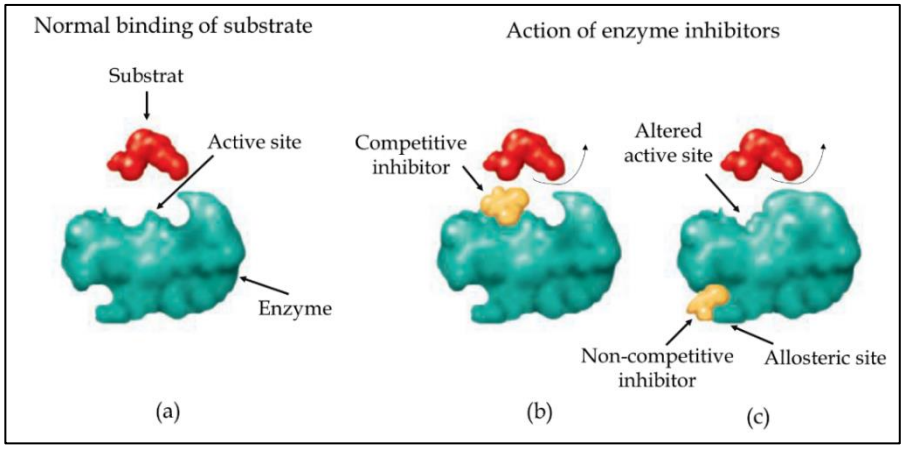


Figure 54. Enzyme inhibitors. **(a):** An uninhibited enzyme and its normal substrate. **(b):** A competitive inhibitor. **(c):** One type of non-competitive inhibitor, causing allosteric inhibition.

3.2. Definition of bacterial metabolism: Catabolism and anabolism

Bacterial metabolism refers to all chemical reactions within bacterial cells to maintain life, grow, and reproduce. There are two main processes involved in metabolism: catabolism and anabolism. Catabolism breaks down substances to create energy and reduce chemical compounds, while anabolism is the process by which bacteria use energy and reduced substances to produce large molecules in the cells.

3.2.1. Bacterial catabolism of carbohydrates

Carbohydrates are the primary energy source for bacteria. Catabolism involves three main pathways: glycolysis, the Krebs cycle, and the electron transport chain (ETC), collectively termed cellular respiration.

a) Glycolysis (The Embden-Meyerhof Pathway)

Glycolysis, also known as the Embden-Meyerhof Pathway (EMP), is the most prevalent glycolytic pathway in both eukaryotic and bacterial organisms. This anerobic (oxygen-independent) process takes place in the cytoplasm and converts one glucose molecule into two pyruvate molecules, generating two ATP (net) and two NADH per glucose (Figure 55). Glycolysis serves as the foundational metabolic pathway for both aerobic respiration and fermentation. The following is a summary of the essential processes:

- **Phase 1 (Preparatory phase):** The enzyme glucokinase or hexokinase phosphorylates glucose, forming glucose-6-phosphate (G6P). Phosphoglucose isomerase converts G6P to fructose-6-phosphate (F6P). The enzyme phosphofructokinase-1 (PFK-1) phosphorylates F6P to fructose-1,6-bisphosphate (F1,6BP). Two ATP molecules are consumed.

- **Phase 2 (Energy-generating phase):** Aldolase cleaves F1,6BP into two 3-carbon molecules, namely dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G3P). G3P is produced from DHAP by triosephosphate isomerase. Glyceraldehyde-3-phosphate dehydrogenase converts G3P to 1,3-bisphosphoglycerate (1,3BPG), which subsequently produces NADH. The enzyme phosphoglycerate kinase converts 1,3BPG into 3-phosphoglycerate (3PG), also forming ATP. Phosphoglycerate mutase converts 3PG into 2-phosphoglycerate (2PG). Enolase dehydrates 2PG, forming phosphoenolpyruvate (PEP). Pyruvate Kinase converts PEP into pyruvate, which is the final step in producing ATP.

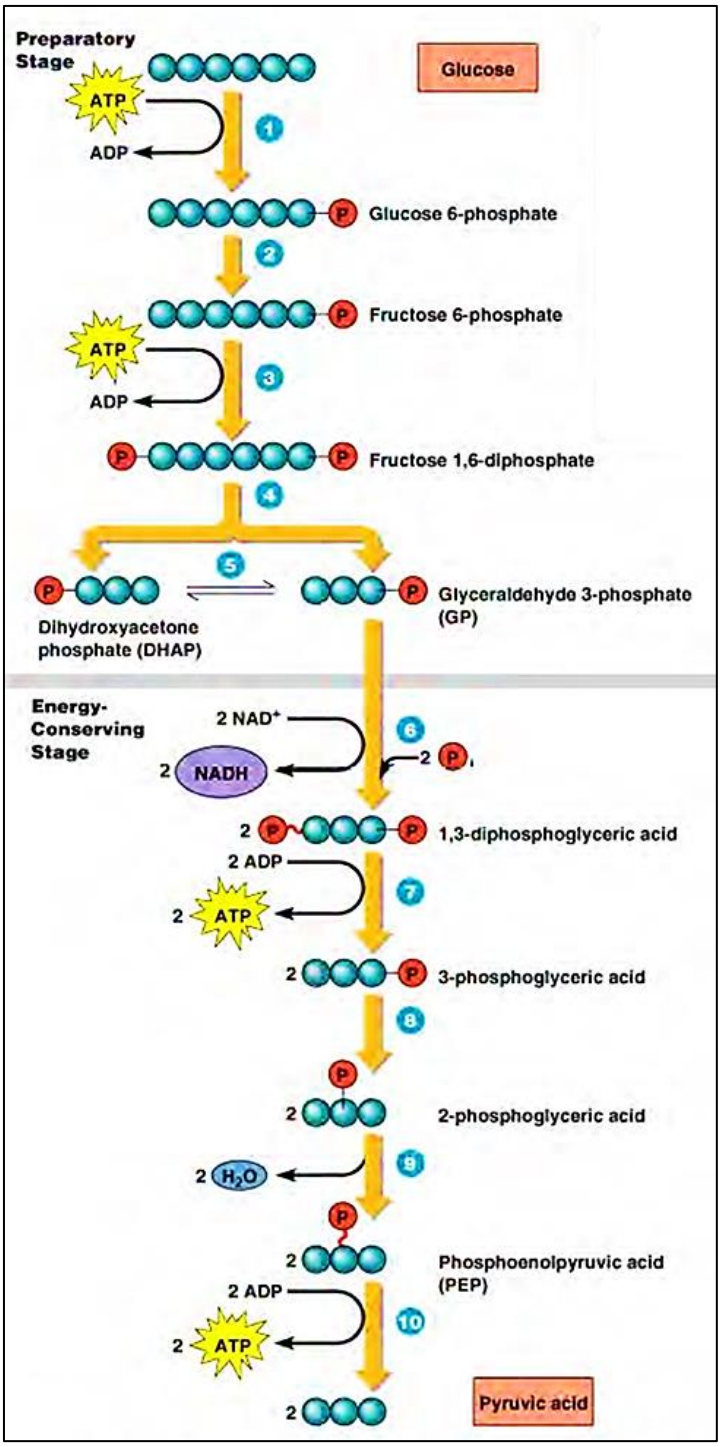


Figure 55. The glycolysis (The Embden-Meyerhof Pathway).

The overall yield of this process is two ATP molecules per glucose molecule, even though four ATP molecules are created, two of which are lost in previous steps. The overall reaction is as follows:

$$\mathbf{Glucose + 2ADP + 2P_i + 2NAD^+ \rightarrow 2Pyruvate + 2ATP + 2NADH + 2H^+ + 2H_2O}$$

b) Alternative pathways: The Entner-Doudoroff (ED) pathway

This pathway represents an alternative to the EMP pathway for glucose metabolism in bacteria. Bacteria that have the enzymes for the ED pathway can metabolize glucose without either glycolysis or the pentose phosphate pathway. The ED pathway is found in some Gram-negative bacteria, including *Rhizobium*, *Pseudomonas*, and *Agrobacterium*. It is generally not found among Gram-positive bacteria. This pathway provides several advantages, including a lower enzyme requirement than the EMP route, and produces different by-products compared to glycolysis. From each glucose molecule, this pathway produces two pyruvate molecules, one ATP molecule and two NADPH molecules for use in cellular biosynthetic reactions (Figure 56).

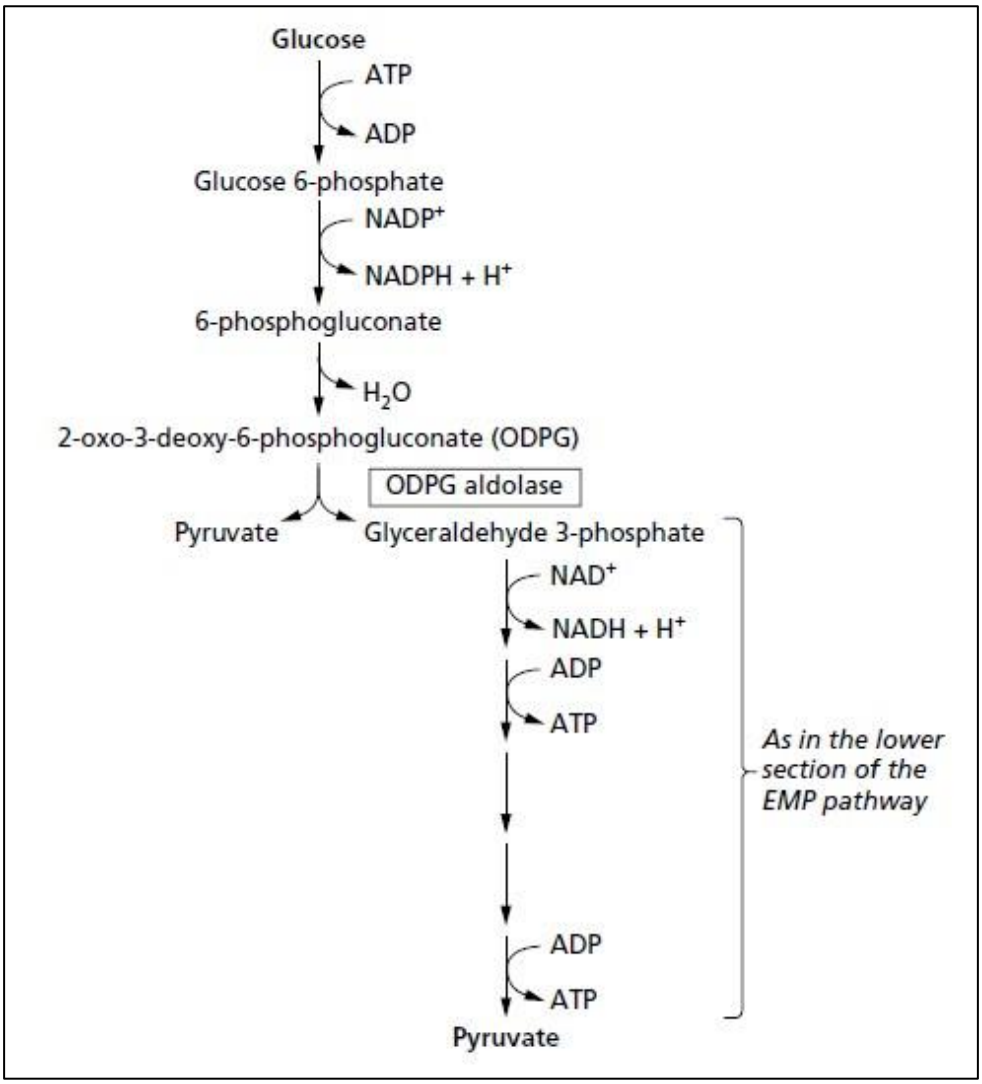


Figure 56. The Entner-Doudoroff (ED) pathway.

The main steps of the ED pathways include):

- **Step 1 (Phosphorylation):** The enzyme Glucokinase phosphorylates glucose to glucose-6-phosphate (G6P) using one ATP.
- **Step 2 (First oxidation):** The G6P is oxidized to 6-phosphogluconate by Glucose Dehydrogenase, generating NADPH.
- **Step 3 (Dehydration):** The 6-phosphogluconate is dehydrated to form 2-keto-3-deoxy-6-phosphogluconate (KDPG) by the 6-phosphogluconate dehydratase.
- **Step 4 (Splitting):** KDPG aldolase splits molecule KDPG in pyruvate (first end product) and G3P, which continues to the next step.
- **Step 5 (Final conversion):** Glyceraldehyde-3-phosphate dehydrogenase oxidizes G3P to 1,3-bisphosphoglycerate (1,3BPG), generating NADH. G3P is converted through the same steps as in glycolysis.

c) Alternative pathways: The Pentose phosphate pathway (PPP)

The pentose phosphate pathway (PPP), also referred to as the hexose monophosphate shunt or phosphogluconate pathway, represents an alternative process for glucose metabolism that operates in parallel to glycolysis to break down pentoses as well as glucose and produce NADPH for biosynthesis (Figure 57). Bacteria that use the PPP include *Bacillus subtilis, E. coli, Leuconostoc mesenteroides*, and *Enterococcus faecalis*.

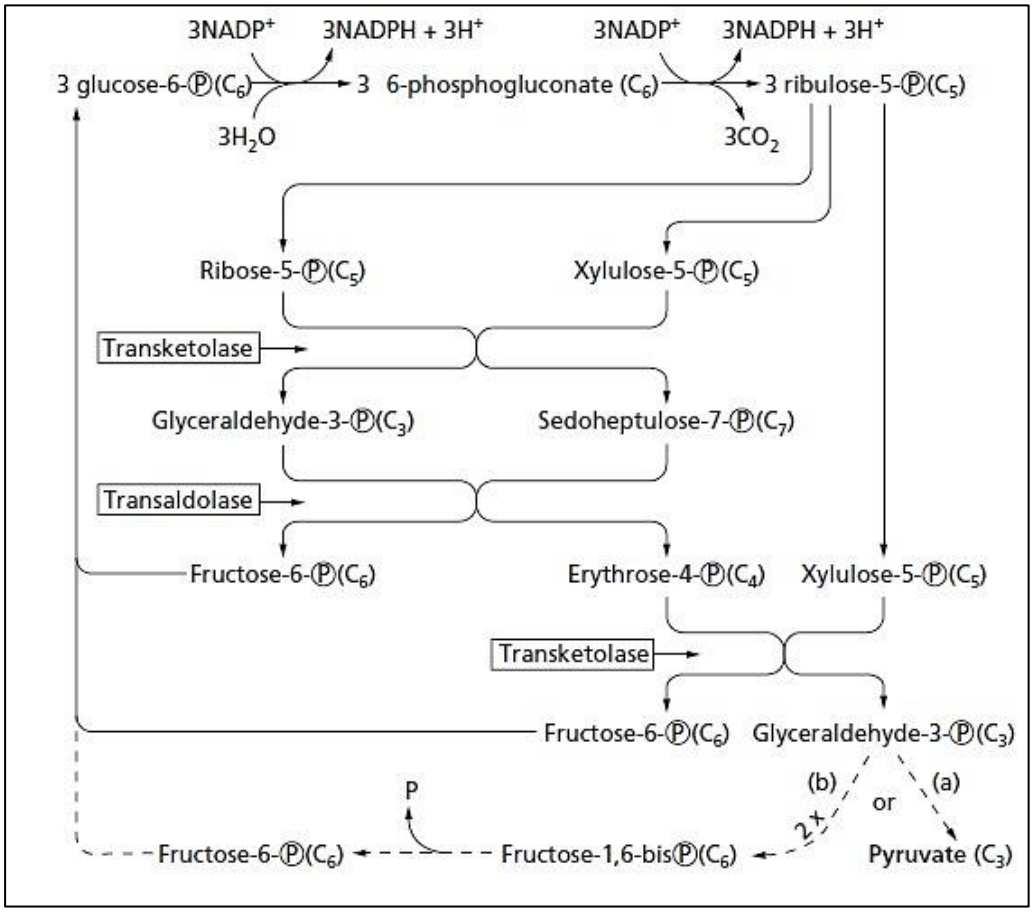


Figure 57. The pentose phosphate (PPP) pathway.

The metabolic reactions involved in the PPP pathway are divided into two distinct phases: oxidative and non-oxidative.

- **Oxidative phase:** The enzyme glucose-6-phosphate dehydrogenase (G6PDH) oxidizes glucose-6-phosphate is to 6-phosphogluconate (6-PG). 6-PG is further oxidized to ribulose-5-phosphate (R-5-P) by 6-phosphogluconate dehydrogenase (6PGDH).
- **Non-oxidative phase:** The key intermediates include R-5-P, sedoheptulose-7-phosphate, and erythrose-4-phosphate (Figure 58).

The PPP pathway confers several advantages upon bacteria, including:

- ✓ The generation of NADPH from $NADP^+$, which is essential for biosynthetic reactions and maintaining cellular redox balance.
- ✓ The PPP yields a net gain of only one molecule of ATP for each molecule of glucose oxidized.
- ✓ The PPP pathway provides ribose-5-phosphate, an essential precursor for nucleotide synthesis.
- ✓ The PPP plays a role in maintaining carbon balance within the cell (carbon homeostasis).
- ✓ The non-oxidative phase of the PPP can generate glycolytic intermediates (fructose-6-phosphate and glyceraldehyde-3-phosphate), which can feed back into glycolysis.

d) Respiration: The Krebs cycle

The Krebs cycle, also called tricarboxylic acid (TCA) cycle or citric acid cycle, is a fundamental metabolic route in bacterial carbohydrate catabolism. This circular circuit is essential for energy generation and the synthesis of cellular components. It occurs in the cytoplasm of prokaryotic cells and fulfils two primary functions:

- ✓ **Energy generation:** Generates reduced electron carriers (NADH and $FADH_2$) that feed into the electron transport chain for ATP synthesis.
- ✓ **Biosynthesis:** Supplies precursors for the synthesis of amino acids, nucleotides, and other biomolecules.

The primary steps of the Krebs cycle (Figure 58) are as follows:

- **Citrate formation:** Citrate synthase catalyzes the condensation of acetyl-CoA (2 carbons) and oxaloacetate (4 carbons) to produce citrate (6 carbons).
- **Isomerization:** Aconitase converts citrate to isocitrate via a sequence of stages.
- **Oxidative decarboxylation:** Isocitrate dehydrogenase catalyzes the oxidation and decarboxylation of isocitrate to produce α-ketoglutarate, releasing CO_2 and $NADH_2$.
- **Succinyl-CoA formation:** The α-Ketoglutarate dehydrogenase complex catalyzes the conversion of α-ketoglutarate to succinyl-CoA, releasing an additional CO_2 and $NADH_2$.
- **Succinate formation:** Succinyl-CoA synthetase converts succinyl-CoA to succinate, concomitant with the production of GTP (or ATP in some bacteria).

- **Fumarate formation:** Succinate dehydrogenase catalyzes the oxidation of succinate to fumarate, yielding $FADH_2$.
- **Malate synthesis:** Fumarase hydrates fumarate to generate malate.
- **Oxaloacetate regeneration:** Malate dehydrogenase oxidizes malate to oxaloacetate, yielding NADH and concluding the cycle.

The Krebs cycle generates 3 NADH molecules, 1 $FADH_2$ molecule, and 1 GTP (or ATP) molecule. The reduced electron carriers (NADH and $FADH_2$) enter the electron transport chain, ultimately leading to the production of ATP via oxidative phosphorylation.

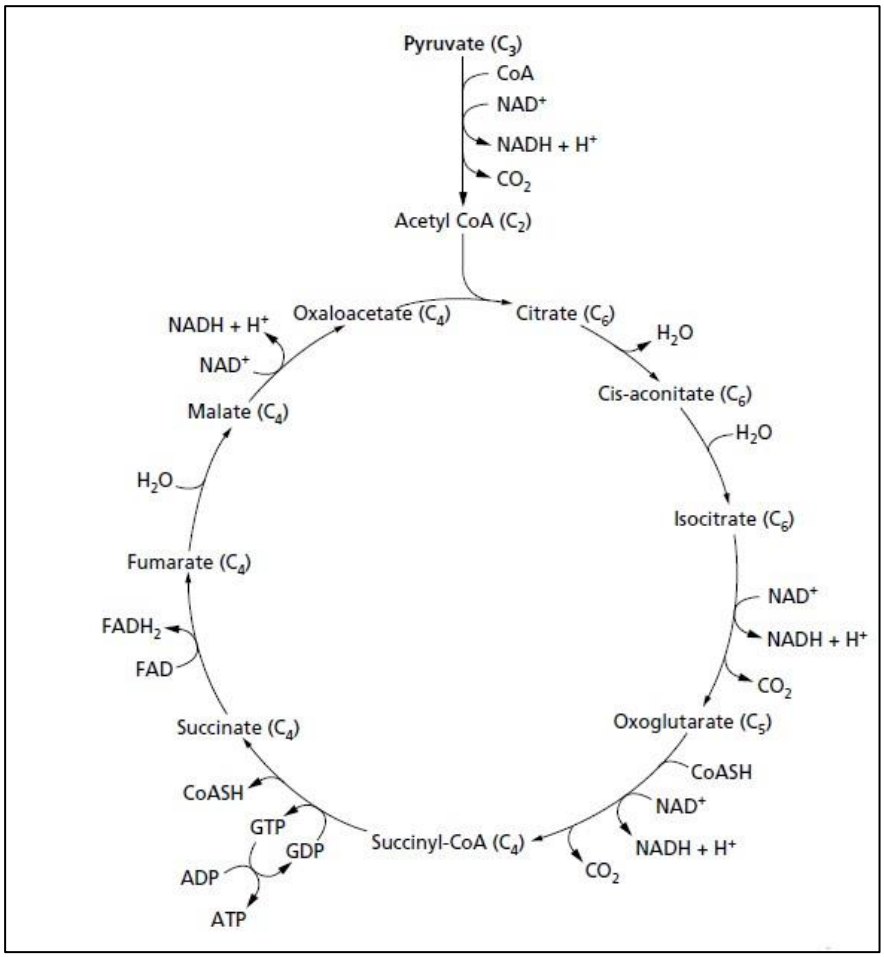


Figure 58. The Krebs cycle.

e) Respiration: The electron transport chain (ETC) and oxidative phosphorylation

- **Electron transport chain (ETC)**

It is a series of protein complexes and other molecules embedded in the bacterial cytoplasmic membrane. Its primary function is to generate a proton gradient across the membrane by transferring electrons from electron donors (e.g. NADH, $FADH_2$) to a terminal electron acceptor (e.g. oxygen) via redox reactions. This gradient is then used to produce ATP during oxidative phosphorylation (Figure 60). The key components of the ETC in bacteria are:

- ✓ **Electron donors:** NADH and $FADH_2$ generated during glycolysis, the citric acid cycle, and other metabolic pathways provide high-energy electrons.
- ✓ **Electron carriers:** (i) Flavoproteins, proteins that contain a flavin group (e.g., FMN) and accept electrons from NADH; (ii) Quinones (e.g., ubiquinone), lipid-soluble molecules that transfer electrons between complexes; (iii) Cytochromes, proteins containing heme groups that carry electrons via the iron atom.
- ✓ **Protein complexes:** These are embedded in the plasma membrane and are involved in electron transfer. Examples include NADH dehydrogenase (Complex I) and cytochrome oxidase (Complex IV).

- **Oxidative phosphorylation**

The oxidative phosphorylation is the final step of bacterial cellular respiration, occurring within the cytoplasmic membrane. It is the process in which the energy from electrons, transferred via the ETC within the cytoplasmic membrane, is used to produce ATP via ATP synthase. This multi-subunit enzyme complex uses the energy from the proton (H^+) gradient (proton motive force, PMF) to catalyze the phosphorylation of ADP to ATP (Figure 59). The energy yield is 3 ATP molecules per NADH molecule and 2 ATP molecules per $FADH_2$ molecule.

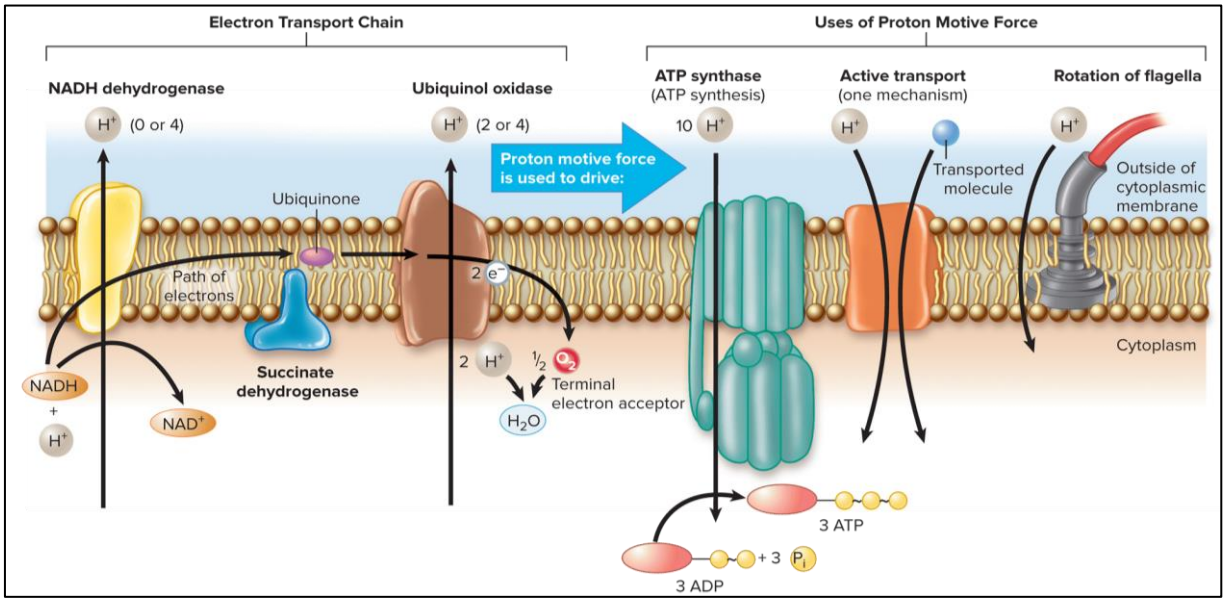


Figure 59. Electron transport chain (ETC), proton motive mortice, and ATP generation.

d) Fermentation

Fermentation is a metabolic process that releases energy from sugars or other organic molecules (e.g., amino acids, organic acids, purines, pyrimidines). It is an anaerobic ATP-generating process where organic compounds act as both electron donors and acceptors, with no external acceptors, such as O_2. It utilizes internally generated electron acceptors derived from the original substrate, unlike respiration, which relies on external electron acceptors.

Fermentation produces small amounts of ATP (1–2 ATP per molecule of substrate) because most of the energy remains in the chemical bonds of end-products like lactic acid or ethanol. It does not involve the Krebs cycle or an electron transport chain. Key Features include:

- ✓ During fermentation, electrons (and protons) from reduced coenzymes (NADH, NADPH) are transferred to pyruvic acid or its derivatives.
- ✓ The main function of fermentation is to regenerate NAD^+ and $NADP^+$, allowing glycolysis to continue.
- ✓ ATP is generated only during glycolysis via substrate-level phosphorylation.
- ✓ Substrate and End-Products: Microorganisms ferment a variety of substrates, and the specific end-products depend on the microorganism, substrate, and enzymes involved.

Table (3) represents the most common and important types of bacterial fermentation.

Table 3. Common types of bacterial fermentation.

Type	Key bacteria	Products	ATP	Applications
Homolactic	*Lactobacillus acidophilus*	2 Lactate	2 ATP	Yogurt, cheese, probiotics
Heterolactic	*Leuconostoc mesenteroides*	Lactate + ethanol + CO_2	1 ATP	Sauerkraut, kimchi
Propionic Acid	*Propionibacterium*	Propionate + acetate + CO_2	1–2 ATP	Swiss cheese (holes/flavor)
Butyric Acid	*Clostridium butyricum*	Butyrate + CO_2 + H_2	3 ATP	Biofuels, industrial solvents
Mixed Acid	*Escherichia, Salmonella*	Lactate + acetate + ethanol + H_2 + CO_2	2–3 ATP	Wastewater treatment, plant growth
2,3-Butanediol	*Enterobacter, Bacillus*	Butanediol + ethanol + CO_2	2 ATP	Synthetic rubber, pharmaceuticals

3.2.2. Bacterial catabolism of lipids

Lipids can also be used as a source of energy to produce ATP. The breakdown of lipids involves extracellular enzymes called lipases, which hydrolyze lipids into their constituent components: glycerol and fatty acids. Each component is metabolized separately to generate energy (Figure 60).

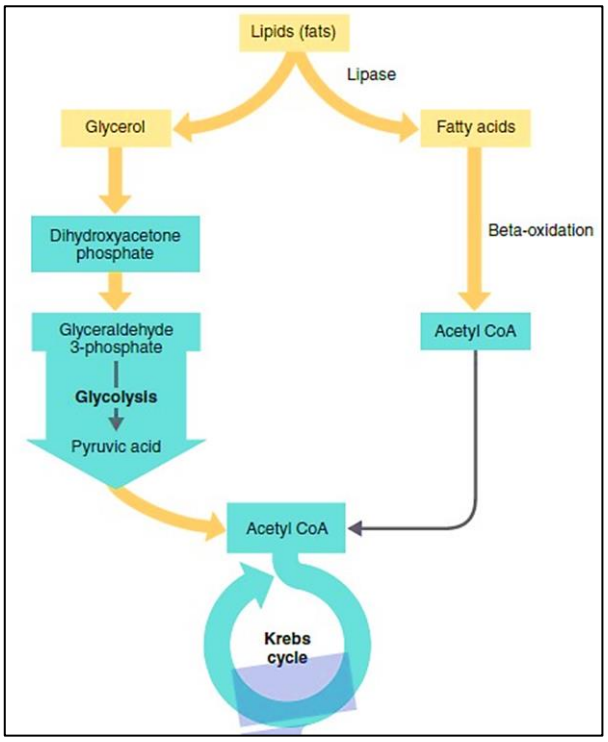


Figure 60. Lipid catabolism.

a) Hydrolysis of triglycerides

- **Lipases**: Catalyze the breakdown of triglycerides into glycerol and three fatty acids through hydrolysis reactions.
- **Location**: Extracellular (secreted lipases) or periplasmic (Gram-negative bacteria).

b) Glycerol catabolism

- **Phosphorylation:** Glycerol is phosphorylated to glycerol- 3-phosphate (G3P) using ATP.
- **Oxidation:** G3P is oxidized to dihydroxyacetone phosphate (DHAP), an intermediate in glycolysis.
- **Integration into glycolysis**: DHAP enters glycolysis at the energy-generating phase, yielding 2 ATP and 1 NADH per glycerol molecule.

c) Fatty acid catabolism: β-oxidation

β-oxidation is the principal pathway for fatty acid degradation, occurring in the cytoplasm of prokaryotes. The key steps of this pathway are:

- **Activation:** Fatty acids are converted to acyl-CoA via acyl-CoA synthetase, consuming 2 ATP.
- **Transport into the matrix:** Acyl-CoA binds to carnitine shuttle proteins (in some bacteria) for transport across membranes.
- **Oxidative cycles:**
  - ✓ Dehydrogenation: Acyl-CoA loses two hydrogens, forming a double bond (producing $FADH_2$).

- ✓ Hydration: Water is added to the double bond.
- ✓ Second dehydrogenation: A hydroxyl group is oxidized to a ketone (producing NADH).
- ✓ Thiolytic cleavage: The fatty acid chain is shortened by two carbons, releasing acetyl-CoA

d) Energy yield

The energy derived from lipid catabolism is significant because fatty acids contain a high number of carbon-hydrogen bonds. Each cycle of β-oxidation produces 1 acetyl-CoA, 1 NADH, and 1 $FADH_2$, which feed into the electron transport chain to generate ATP.

e) Applications

Lipid catabolism in bacteria presents ecological and industrial relevance. For example, *Pseudomonas* spp. degrades petroleum hydrocarbons via β-oxidation, critical for bioremediation.

3.2.3. Bacterial catabolism of proteins

Proteins are large macromolecules that cannot pass through plasma membranes unaided. Microorganisms produce extracellular enzymes such as proteases and peptidases, which break proteins down into smaller peptides or amino acids. These smaller molecules can then cross the cell membrane and enter metabolic pathways.

a) Proteolysis

- ✓ **Proteases and peptidases:** extracellular enzymes (e.g., *B. subtilis*) hydrolyze proteins into oligopeptides and free amino acids.
- ✓ **Transport:** Amino acids are transported into the cell for further processing.

b) Amino acid degradation

Once inside the cell, amino acids undergo various transformations to enter central metabolic pathways:

- **Deamination**: The amino group ($-NH_2$) is removed from amino acids and converted to ammonium ions ($NH4^+$), which are excreted from the cell. The remaining organic acid (keto acids) enters the Krebs cycle. Examples include:
  Glutamate dehydrogense : $\text{Gluatamte} \rightarrow \alpha - \text{ketoglutarate (Krebs cycle intermediate)} + NH_3$
- **Transamination:** e.g., Transfer of ($-NH_2$) to α-ketoglutarate or oxaloacetate. Example include:
  Alanine transaminase: $\text{Alanine} + \alpha - \text{ketoglutarate} \rightarrow \text{pyruvate} + \text{glutamate}$
- **Decarboxylation**: Some amino acids lose their carboxyl group (-COOH), producing amines and CO_2. Examples include:
  Lysine dehydrogenase: $\text{Lysine} \rightarrow \text{Cadaverine (Common in Enterobacteriaceae)}$

c) Sulfur metabolism

Sulfur-containing amino acids, such as cysteine, are metabolized to release hydrogen sulfide (H_2S). For example, cysteine desulfhydrase converts cysteine to pyruvate, releasing H_2S as a

byproduct. H_2S combines with iron in culture media to form black precipitates (ferrous sulfide), which can be used diagnostically (e.g., distinguishing *Salmonella* from *E. coli*).

d) Integration with central metabolism

The organic acids (keto acids), e.g., pyruvate and α-ketoglutarate, produced during protein catabolism feed directly into central pathways, such as glycolysis or the Krebs cycle:

e) Applications

Biochemical tests based on protein catabolism help identify bacterial species. E.g., H_2S production differentiates *Salmonella* from *E. coli*. Additionally, protein catabolism plays a role in food production. For example, *Propionibacterium* converts lactic acid to propionic acid during Swiss cheese fermentation, creating its characteristic holes.

3.2.4. Bacterial anabolism: Peptidoglycan synthesis

Peptidoglycan, a critical component of the bacterial cell wall, is synthesized through a multi-stage process involving cytoplasmic, membrane-bound, and extracellular reactions (Figure 61).

a) Cytoplasmic phase: Precursors synthesis

The biosynthesis initiates in the cytoplasm with the production of two nucleotide-activated amino sugars: uridine diphosphate N-acetylglucosamine (UDP-NAG) and UDP-N-acetylmuramic acid (UDP-NAM). UDP-NAM molecules undergo sequential modification through the addition of five amino acids, forming UDP-NAM-pentapeptides. Specific ligases catalyze the addition of each amino acid, and interestingly, some of these amino acids exist in the D-form (isomers), in contrast to the L-form typically found in proteins. This unique characteristic contributes to the structural stability of peptidoglycan and resistance to proteolytic enzymes.

b) Membrane-bound phase: Lipid carrier assembly

Once the UDP-NAM-pentapeptide is formed, the NAM-pentapeptide is transferred from UDP to a lipid carrier known as bactoprenol phosphate, which is anchored on the inner side of the bacterial cell membrane. This transfer creates a bactoprenol-linked NAM pentapeptide complex. Subsequently, UDP-NAG adds its NAG moiety to this complex, forming a complete subunit (NAG-NAM-pentapeptide) necessary for peptidoglycan assembly.

c) Extracellular phase: Polymerization and cross-linking

The bactoprenol-linked NAG-NAM-pentapeptide unit is transported across the cell membrane to the periplasmic space. There, the NAG-NAM-pentapeptide is released from bactoprenol and incorporated into the incomplete peptidoglycan chain. This process involves two critical steps:

- Transglycosylation: Enzymes catalyze the polymerization of glycan chains by linking NAG-NAM units.
- Transpeptidation: Peptide cross-links are formed between peptidoglycan chains, creating a rigid three-dimensional network. This step is mediated by transpeptidases (penicillin-binding

proteins). Energy for cross-linking is provided by the cleavage of the terminal D-alanine residue in the pentapeptide by carboxypeptidases, as ATP is not available in this extracellular location.

The bactoprenol carrier then returns to the cytoplasmic side of the membrane to participate in another cycle of precursor transport.

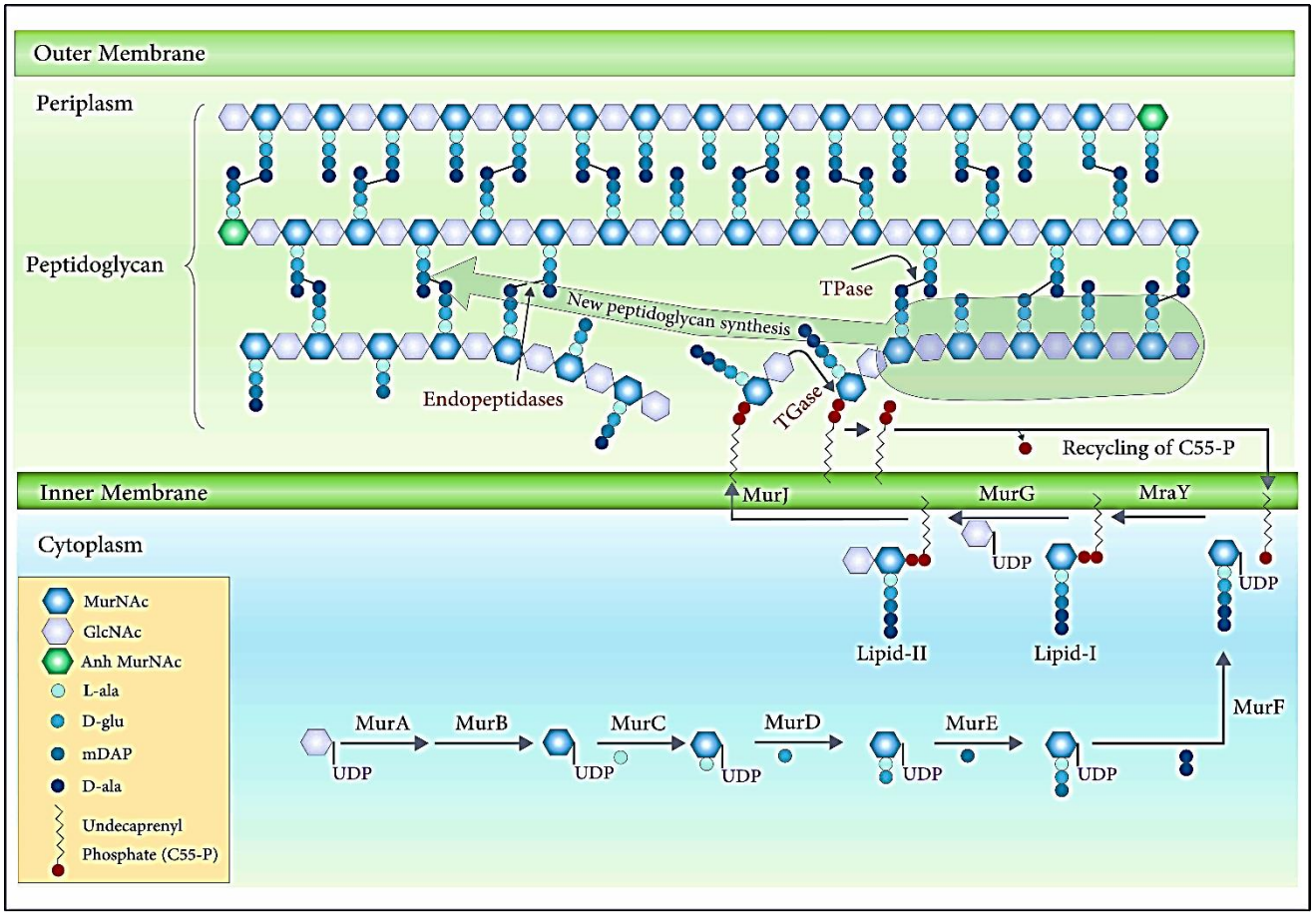


Figure 61. A schematic presentation of the peptidoglycan biosynthesis pathway.

Chapter IV

Bacterial Growth

Chapter IV. Bacterial Growth

Bacterial growth and reproduction are intricately linked processes influenced by various environmental factors.

1. The Concept of Bacterial Growth and Reproduction

The term “growth” often refers to the regular and balanced increase of an organism in size, weight, or number. In multicellular organisms, growth is characterized as an augmentation in the organism's physical dimension, such as length or size. In contrast, in unicellular organisms, such as bacteria, growth is defined as an increase in the cell number in a population or a culture compared to its initial quantity.

The process of bacterial growth is influenced by various environmental factors, including temperature, humidity, acidity, light, aeration, nutrient availability, and the presence of toxic substances, as well as biological interactions, such as competition and parasitism. Accordingly, in optimal environmental conditions, bacteria grow through the following mechanisms:

- ✓ The increase in bacterial cell size: The quantity of the protoplasm increases, resulting in an augmentation of all the cellular components (e.g., DNA, RNA and proteins), thereby increasing the cell mass. Upon reaching a specific size, the cells cease to grow and begin to divide.
- ✓ The increase in the bacterial cell number: There is a reciprocal correlation between the growth rate and cellular division. The greater the increase in cell mass, the greater the cell division, which increases the number of cells. The term "reproduction" biologically denotes the generation of new individuals possessing identical physiological characteristics to their parents.

In the context of unicellular organisms, such as bacteria, division is a reproduction method because it generates two new cells. In contrast, in multicellular organisms like animals and plants, cell division results only in growth (i.e. increase in size). However, in unicellular microorganisms, such as bacteria, the terms “growth” and “reproduction” are synonymous, signifying an increase in the number of cells.

2. Environmental Factors Influencing Bacterial Growth

Bacterial growth and proliferation are significantly influenced by various environmental factors. For bacteria to utilize their nutritional requirements effectively, these nutrients must be available under specific physicochemical conditions, including temperature, pH, osmotic pressure, and other factors.

2.1. Oxygen requirements

Bacteria exhibit diverse O_2 requirements and can be classified into different groups (Figure 62).

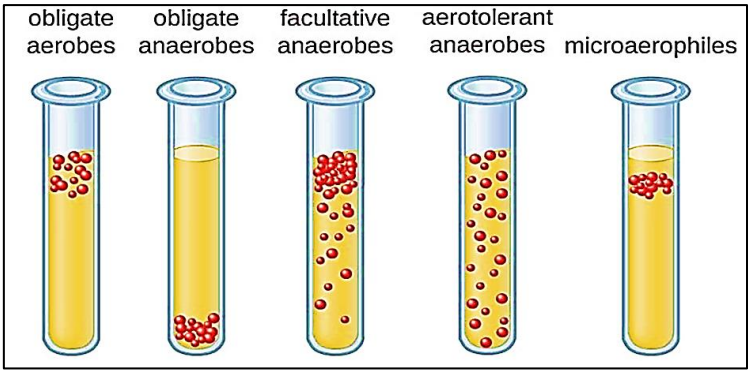


Figure 62. Classification of bacteria based on oxygen requirements.

a) Obligate aerobic bacteria (Obligate aerobes)

They require O2 for growth and cannot grow in its absence. Their primary energy source is aerobic respiration, where O2 serves as the final electron acceptor and is reduced to water. Examples include *Pseudomonas aeruginosa, Acinetobacter*, and *Neisseria*.

b) Facultative anaerobic bacteria (Facultative anaerobes)

They can grow both in the presence and absence of O2 through aerobic respiration (in aerobic conditions) and fermentation or anaerobic respiration (in anaerobic conditions). Examples include members of the Enterobacteriaceae group (e.g., *Escherichia, Salmonella, Klebsiella, Shigella*), *Streptococcus*, and *Staphylococcus aureus*.

c) Obligate anaerobic bacteria (Obligates anaerobes)

They grow in the absence of O_2, which is toxic and lethal to them. Examples include *Clostridium perfringens* and *C. botulinum*.

d) Microaerophilic bacteria (Microaerophiles)

They grow only or optimally in environments with low O_2 concentrations. Examples include *Helicobacter pylori, Campylobacter jejuni*, and *Salmonella typhimurium*.

e) Aerotolerant anaerobic bacteria

They grow and multiply both in the presence and absence of O_2. They exclusively use fermentation for their metabolic processes. Examples include some *Clostridium* species.

2.2. Temperature effects

Temperature is one of the most critical factors affecting bacterial growth. Based on temperature requirements, bacteria can be classified into the following categories:

a) Psychrophiles

They have an optimum growth temperature below 20°C (-10-20°C), with optimal growth at 10-15°C. They die at room temperature (25°C) and are found in consistently cold environments (e.g., polar regions). An example is *Bacillus psychrophilus*.

b) Psychrotrophic bacteria

They can grow at low temperatures like refrigerators, with a temperature range of 0-30°C and optimal growth at around 20°C. Examples include some *Pseudomonas* species.

c) Mesophiles

They grow well between 20-45°C, with optimal growth at 30-45°C. For human and warm-blooded animal pathogens, the optimal temperature is typically 37°C. This group includes bacteria that coexist with humans, saprophytic and pathogenic bacteria. Examples include E. *coli, Salmonella, Neisseria gonorrhoeae*, and *Staphylococcus*.

d) Thermophiles

They thrive at temperatures between 55-80°C, with optimal growth between 55-75°C. Examples include *Bacillus stearothermophilus* and *Thermus aquaticus*.

e) Hyperthermophiles

They grow well at high temperatures above 70°C, with optimal growth between 80-122°C. Examples include *Sulfolobus acidocaldarius* (optimal at 80°C), *Thermococcus celer* (optimal at 88°C), and *Pyrolobus fumarii* (optimal at 106°C, minimum at 90°C).

f) Thermotolerant Bacteria

They can withstand high temperatures, with their vegetative cells able to tolerate and grow at high temperatures, although they grow better at moderate temperatures (e.g., 30°C).

2.3. pH influence

The pH of the environment significantly influences bacterial growth:

a) Neutrophilic bacteria

They prefer to grow in media close to neutral pH (5.5-8.5), with optimal neutral pH (around 7.2-7.6). Most bacteria belong to this group, such as *E. coli, Staphylococcus*, and *Salmonella*.

b) Acidophilic bacteria

They prefer to grow in acidic media (optimal pH less than 5). Examples include *Sulfolobus* (optimal pH 2-3), *Picrophilus, Ferroplasma, Lactobacillus*, and *Thiobacillus thiooxydans*.

c) Alkaliphilic bacteria

They prefer to grow in alkaline media (pH greater than 8), with optimal pH between 8 and 11. Examples include *Alcalophilus, Flavobacterium*, and some *Bacillus* species.

2.4. Water availability and osmotic pressure

a) Water activity (Aw)

Bacteria cannot grow or reproduce normally in dry environments, as water is necessary for their metabolic activities. The availability of free water molecules significantly impacts bacterial growth rates and survival. Water activity (*Aw*) expresses the amount of free water in a medium. The value is high when there are no dissolved substances in the medium water and decreases as the concentration

of dissolved substances (salts, sugars, etc.) increases. For pure water, *Aw* = 1. Most bacteria require *Aw* > 0.9. E.g., *S. aureus* can grow at *Aw* = 0.86, explaining its role in salted food spoilage.

b) Osmotic pressure

Osmotic pressure directly affects bacterial growth through water movement across cell membranes. Based on salt tolerance, we can distinguish the following bacterial groups:

- **Halophilic bacteria:** Require salt to grow and multiply. They include extreme halophiles (15-30% salt, e.g., *Halobacterium*), moderate halophiles (optimal growth at 3-15% salt), and slight halophiles (1-3% salt). They live in seas, oceans, highly saline lakes, dried water pools, and saline soil.
- **Halotolerant bacteria:** These do not require specific salt concentrations for growth but can tolerate and grow in their presence (e.g., 10% salt). Examples include *Staphylococcus*.
- **Non-halophilic bacteria:** These cannot survive even in dilute salt concentrations, and most cannot live in seawater.

3. Binary Fission Process

Binary fission is the primary mode of reproduction in bacteria, enabling exponential population growth under favorable conditions. The process is delineated as follows:

a) Cell growth

The parent cell undergoes biosynthetic expansion, increasing its cytoplasmic volume (protoplasm) and replicating cellular components (ribosomes, enzymes, etc.).

b) DNA replication

The circular bacterial chromosome replicates bidirectionally from the origin of replication. Plasmids (extrachromosomal DNA) replicate independently, often via a rolling-circle mechanism.

c) Chromosome segregation

Replicated genetic material is actively partitioned to opposite cell poles.

d) Cell elongation

The cell grows in size as the chromosomes move to opposite poles. This elongation is particularly evident in rod-shaped bacteria (bacilli) (Figure 63).

e) Septum formation

A septum (transverse cytoplasmic membrane and cell wall) is established, creating a transverse septum.

f) Cytokinesis and cell separation

The septum constricts, separating the cytoplasm into two daughter cells that possess identical characteristics to the original cell.

f) Post-Division

Daughter cells may remain attached (e.g., *Staphylococcus* clusters) or separate fully (e.g., *E. coli*).

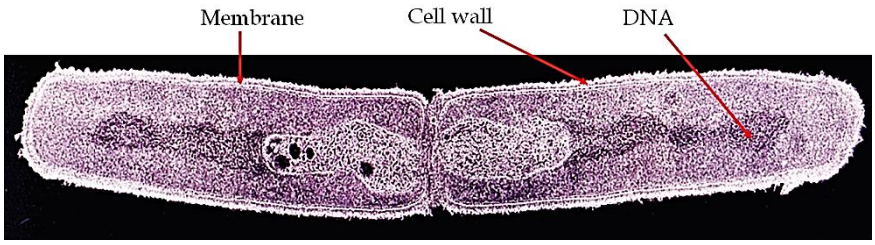


Figure 63. Electron micrograph of *Bacillus subtilis*, a Gram-positive bacteria, dividing by binary fission (Magnification 31200 ×).

4. Bacterial Growth in Batch Culture: Phases of The Bacterial Growth

Bacterial growth in batch culture is characterized by distinct phases that reflect changes in population dynamics and cellular activity. These phases are crucial for understanding microbial physiology and optimizing biotechnological processes. The phases typically include the lag, exponential, stationary, and death phases (Figure 64). Here is a detailed exploration of these phases:

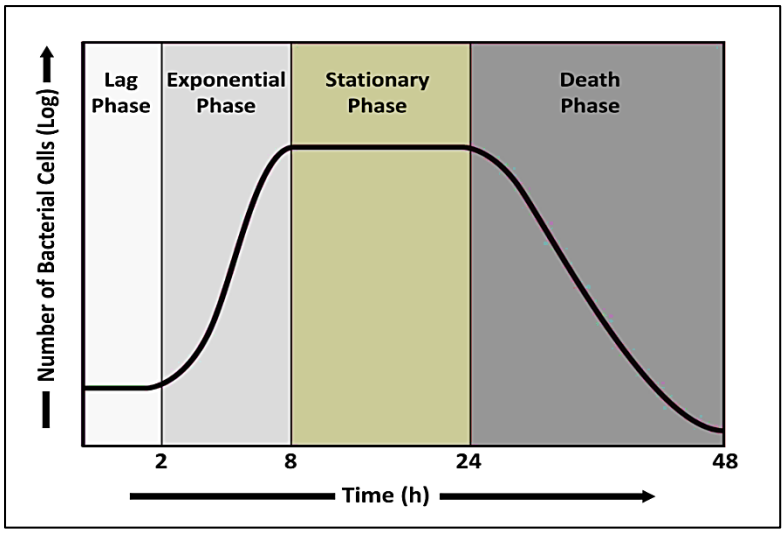


Figure 64. Bacterial growth curve in a batch culture.

4.1. The lag phase

The initial phase observed under batch conditions is the lag phase, during which the growth rate is essentially zero (Figure 64). Once an inoculum has been placed into a fresh medium, the growth process begins after a duration known as the lag phase. During this phase, bacteria adapt to their new environment, and no cell division occurs, although cells undergo intensive metabolic activity in preparation for subsequent division (metabolically active). The lag phase involves the synthesis of enzymes, transport proteins, cofactors, vitamins, ribosomes, and other essential molecules and cell components necessary for growth. The lag phase represents a period of physiological adaptation and biosynthesis that precedes active cell division.

- **Factors influencing the lag phase duration:** The lag phase usually lasts from minutes to several hours. The lag phase length can be controlled because it is dependent on various factors, including:

a) **Nutritional composition of the medium:** The transition of bacteria from a nutrient-rich to a nutrient-deficient medium prolongs this phase due to the additional time needed for bacterial adaptation to the new environment. In contrast, transitioning from a nutrient-deficient to a nutrient-rich medium facilitates rapid adaptation, resulting in a shorter lag phase.

b) **Physiological state of the inoculated culture:** Due to its active metabolic state, the lag phase duration is shorter when the culture originates from a logarithmic (exponential) phase. Conversely, the duration is extended if the culture is inoculated from a stationary phase or a spore-forming state.

c) **Environmental conditions:** The lag period is reduced as the temperature approaches the optimal level for growth. Variations from this optimal temperature, whether an increase or decrease, result in an extended lag phase. Additional parameters, including pH and oxygen, can significantly influence the duration of the lag phase.

4.2. Logarithmic (Exponential) growth phase

The logarithmic (log) phase, also known as the exponential phase, is the second phase observed in a batch culture (Figure 64). This phase is characterized by cells dividing at a constant and maximum rate (μmax), resulting in a rapid increase in population size. It is defined by optimal conditions with abundant nutrients and favorable environmental factors, allowing bacteria to achieve their μmax. This phase continues until nutrient depletion occurs, leading to a cessation of growth and transition into the stationary phase. There are several ways to express exponential growth, both theoretically and mathematically. Under optimal conditions, bacterial cell numbers increase in a geometric progression (2^0, 2^1, 2^2, 2^3, etc.). After n divisions, the total number of cells is expressed as 2^n (Figure 65).

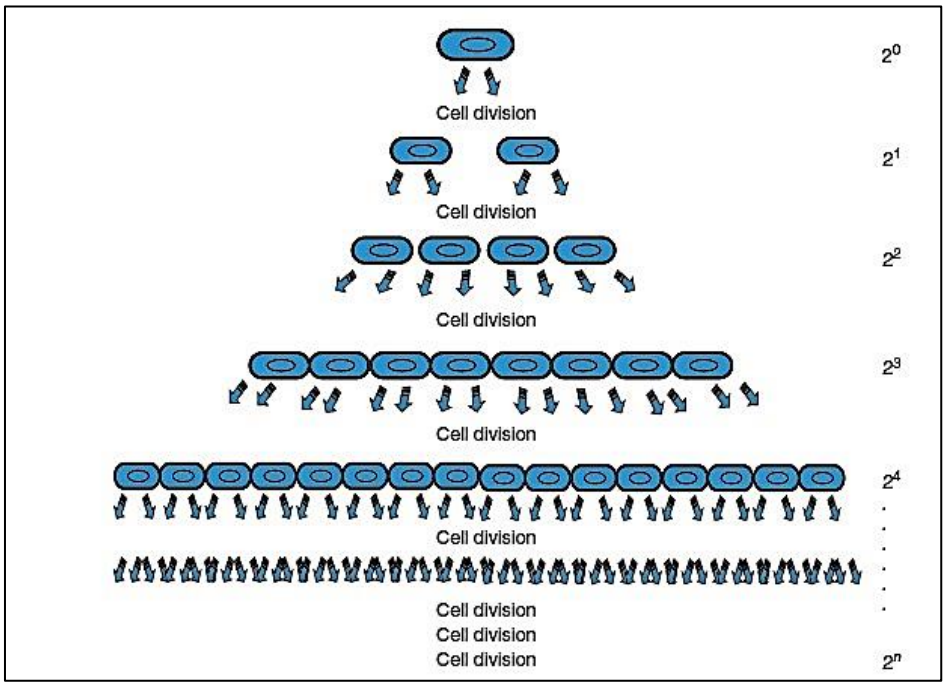


Figure 65. Exponential cell division.

Different bacterial species exhibit varying reproduction rates in culture; therefore, the term "generation time" is used.

a) Generation time (G)

Generation time (G) refers to the duration required for a bacterial cell to undergo division, resulting in two cells, or to double the cell number within a specified timeframe. It can alternatively be defined as the duration that elapses between two consecutive divisions (doubling time).

Generation time can differ between different species and is influenced by environmental factors such as medium composition, temperature, and pH. In optimal environmental conditions, the generation time for *E. coli* is 20 minutes (at 37°C in a nutrient-rich medium), 13 minutes for *Vibrio parahaemolyticus*, 32 minutes for *S. aureus*, and 100 minutes for *Lactobacillus acidophilus*. However, the generation time for *M. tuberculosis* is 1000 minutes.

The generation time is determined during the exponential phase of the bacterial growth curve. If a proliferating bacterial culture starts with (N_0) cells, the number of cells (N) at the end of the experimental duration (t), following n divisions, is expressed as ($N = N_0 \times n^2$). The numerical value can be converted to a logarithmic value, as demonstrated in the following equation:

$\mathrm{LogN} = \mathrm{LogN_0} + \mathrm{nLog2}$ (Eq. 1); $\mathrm{n} = \frac{\mathrm{Log\,N - Log\,N_0}}{\mathrm{Log2}}$ (Eq. 2)

The (G) can be expressed using the following equation: $G = \frac{t}{n}$ (Eq. 3)

Where: t = Growth time of the culture; n = the number of divisions (number of generations)

Example of Generation time calculation

Consider a bacterial culture grown from 10^3 cells/ml (N_0) to 10^9 cells/ml (N) over a duration (t) of 10 hours.

- Calculate number of generations (*n*):

Use the (Eq. 2): n = (Log 10^9 - Log10^3)/Log2 = 20 generations.

- Calculate generation time (*G*):

Use the (Eq. 3), G = 10/20 generations = 0.5 hour, or 30 minutes.

b) Characteristics of the exponential phase

- **Maximum growth rate:** Bacteria proliferate at their maximum specific growth rate (*μmax*) under optimal environmental conditions.
- **Constant doubling time:** The population doubles at regular intervals due to stable environmental conditions.
- **Nutrient utilization:** Bacteria preferentially metabolize simpler carbon sources to sustain rapid growth.

c) Factors influencing the exponential phase

- **Nutrient availability:** The presence of essential nutrients in sufficient quantities is crucial for sustaining exponential growth.
- **Environmental conditions:** Factors such as temperature, pH, and oxygen levels must be optimal to support the high metabolic activity during this phase.
- **Substrate concentration:** High substrate concentrations can support maximum growth rates, although such conditions are rare in natural environments.

4.3. Stationary phase

The stationary phase is the third phase of bacterial growth in batch culture, characterized by a plateau in the bacterial population (Figure 64). Although there is no net increase in population during the stationary phase, cells still grow and divide with growth being balanced by an equal number of cells dying. In this phase, the cellular growth rate slows down due to nutrient depletion, and waste accumulation, and environmental conditions become less favorable for growth, balancing the rate of cell division and death. The stationary phase is particularly important for industrial applications where maintaining high cell density is crucial for production efficiency.

- **Factors influencing the stationary phase**

a) Nutrient depletion: It is the primary factor leading to the stationary phase, as bacteria consume available nutrients, the growth rate slows down, leading to the stationary phase.

b) Accumulation of toxic metabolic by-products: Including volatile compounds that affect bacterial growth. Eliminating these toxins can prolong the stationary phase and delay the death phase.

c) pH changes: The pH of the culture medium may shift during growth, which could potentially affect growth rates at extreme pH levels.

d) Genetic and physiological adaptations: Bacteria undergo genetic adaptations during the stationary phase, such as the expression of the growth advantage in the stationary phase (GASP) phenotype, which allows them to survive under nutrient-limited conditions (increased stress tolerance and survival mechanisms).

e) Environmental stress and intracellular damage: Environmental stressors, including oxidative stress, can induce bacterial dormancy (a survival mechanism), affecting their ability to regrow when conditions improve. Intracellular damage control, including the upregulation of chaperones (e.g., DnaK) to protect proteins, enhancement of DNA repair enzymes to maintain genetic integrity, and the activation of oxidative stress response pathways. These mechanisms are vital for maintaining cell viability during prolonged stationary phases.

4.4. Decline or death phase

It represents the final phase of bacterial growth in a batch culture characterized by a progressive decline in viable cell numbers due to death, which parallels the increase observed during the log phase (Figure 64). The death phase often shows a biphasic pattern, with an initial rapid decline followed by a slower death rate. A few highly resistant organisms persist for an indeterminate duration, influenced by their stress response mechanisms and genetic variations.

The decline phase typically occurs due to nutrient depletion, accumulation of toxic metabolites, and environmental stresses, resulting in a significant reduction in the population of viable cells. Theoretically, the entire population should die at a time interval equivalent to that of the log phase.

5. Bacterial Growth in Continuous Culture

5.1. Definition of continuous culture

Continuous culture is an "open" system used for cultivating microorganisms or cells under steady-state conditions. In this system, fresh sterilized medium is continuously introduced into a bioreactor at a constant flow rate, while an equal volume of culture fluid—containing leftover nutrients, metabolic byproducts, and cells—is simultaneously removed. This maintains a constant culture volume and allows bacteria to remain in the exponential growth phase indefinitely.

The vessel used for continuous culture is called a bioreactor, with the most common type being the chemostat.

5.2 Chemostat

A chemostat (short for "chemical environment is static") is a specialized bioreactor designed to maintain continuous bacterial growth under controlled conditions. It operates as an open system where:

- ✓ Fresh sterilized medium containing nutrients flows into the reactor at a constant rate.
- ✓ An equal volume of culture liquid exits the reactor, maintaining a stable culture volume.

This setup ensures that environmental factors such as pH, oxygen levels, temperature, and nutrient concentrations remain constant, creating reproducible growth conditions.

a) Principle

The chemostat functions by controlling bacterial growth through the limitation of a single essential nutrient (e.g., carbon or nitrogen). While other nutrients are provided in excess, this limiting nutrient determines the growth rate. By adjusting the dilution rate, at which fresh medium is added and culture liquid is removed, researchers can:

- ✓ Control the bacterial growth rate.
- ✓ Achieve a steady state where cell division matches the dilution rate.

At steady state:

- The bacterial population remains constant.
- The growth rate equals the dilution rate.
- Cells remain in exponential growth indefinitely under optimal conditions.

Continuous stirring ensures uniform mixing within the reactor, preventing nutrient gradients and maintaining homogeneity.

b) Applications of chemostats

Chemostats have diverse applications in microbiology and biotechnology:

- **Biotechnology:** Production of antibiotics (e.g., penicillin), enzymes (e.g., amylase), or biofuels under controlled conditions.
- **Environmental applications:** Used in wastewater treatment plants to degrade organic pollutants efficiently.
- **Microbial evolution studies:** Chemostats allow researchers to study adaptive evolution by exposing microorganisms to selective pressures over extended periods. They are also used to investigate resistance mechanisms against antimicrobial agents.
- **Reproducible research:** Chemostats provide consistent conditions for studying microbial physiology, metabolism, and interactions.

6. Estimation of Bacterial Growth

The estimation of bacterial growth is a fundamental aspect of microbiology, allowing the quantification of bacterial populations under various conditions. This process is essential for studying bacterial physiology, monitoring microbial activity, and assessing contamination in different environments.

There are two main approaches to estimating bacterial growth: measuring the number of cells and determining the cell mass.

6.1. Estimation of cell number

6.1.1. Plate count method

The plate count method is a standard technique used to estimate the number of viable bacterial cells in a sample. It involves several steps:

- **Serial Dilutions:** The original sample is diluted in a series of steps to reduce the concentration of bacteria. For example, a 1 mL sample may be diluted into 9 mL of sterile diluent to create a 10-fold dilution (10^{-1}). This process is repeated to achieve further dilutions, such as 10^{-2},10^{-3}, and so on (Figure 66).

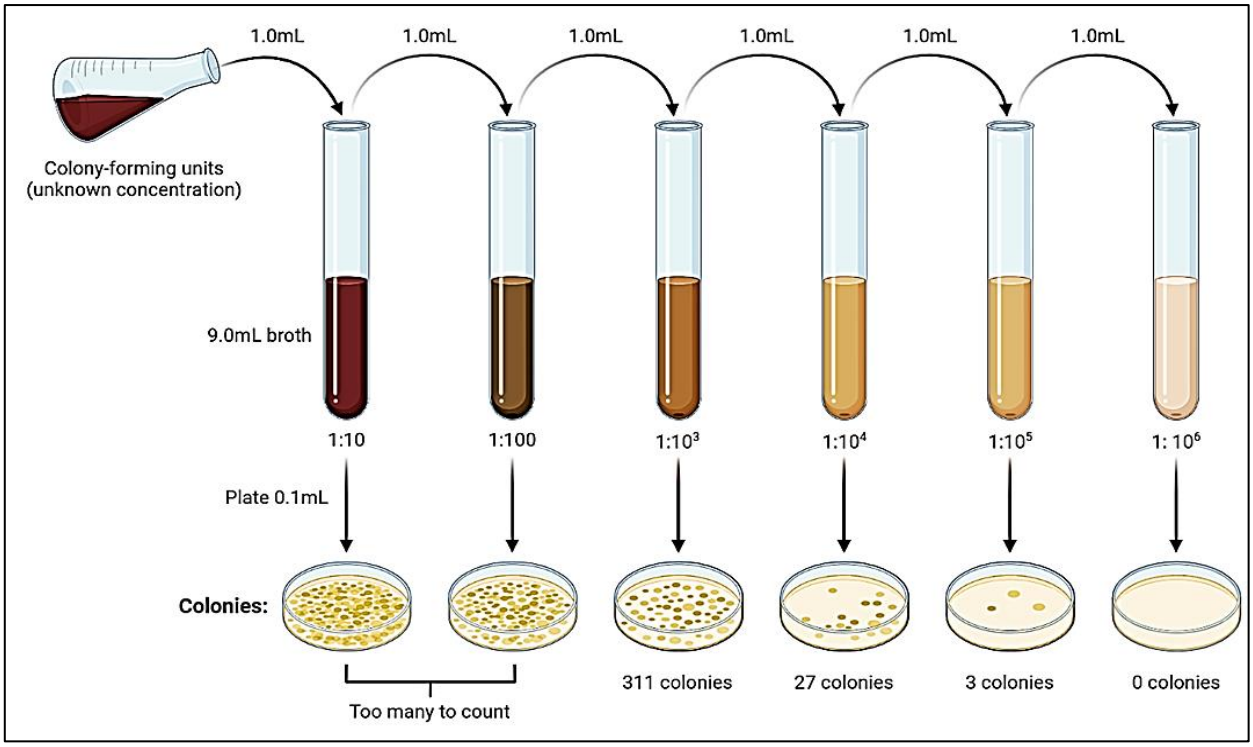


Figure 66. Plate count method.

- **Plating Techniques:** In the spread plate method, a small volume (e.g., 0.1 mL) from each dilution is spread evenly over the surface of an agar plate (Figure 6). In the pour plate method, a measured volume (e.g., 1 mL) from each dilution is mixed with molten agar and poured into a sterile Petri dish (Figure 67).

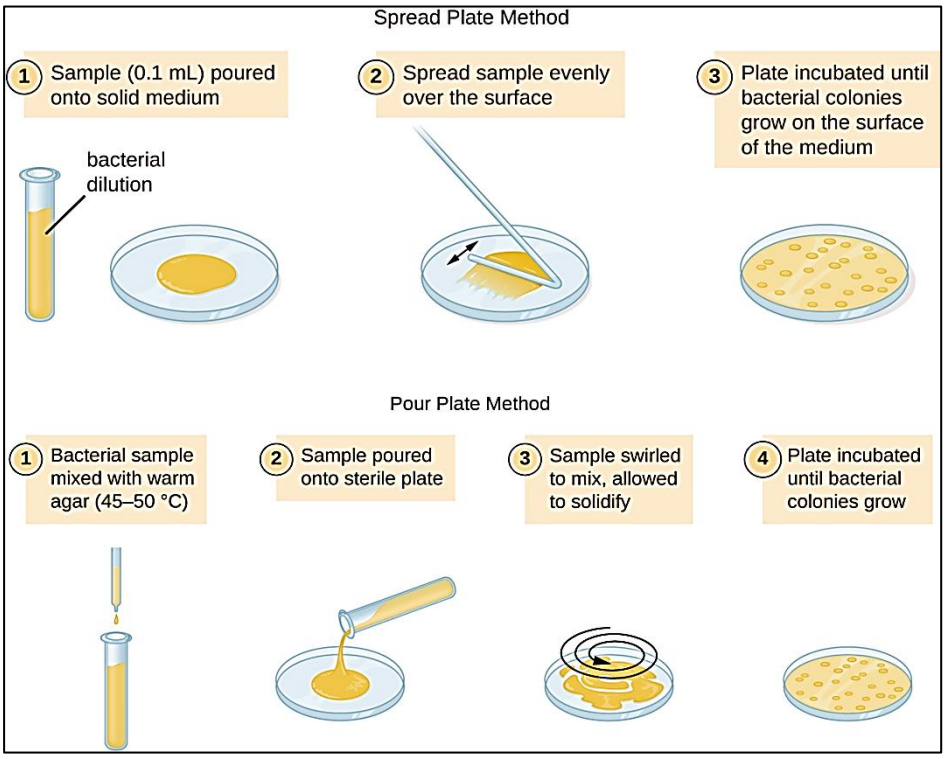


Figure 67. Plating techniques: The spread plate and the pour plate methods.

- **Incubation:** The plates are incubated under optimal conditions (e.g., temperature and oxygen requirements) until visible colonies form.
- **Colony counting:** After incubation, plates containing between 30 and 300 colonies are selected for counting. This range ensures statistical reliability and minimizes errors due to overlapping colonies or insufficient growth.
- **Calculations:** The concentration of bacteria in the original sample is calculated using the following formula:

$$\text{CFU/mL or g} = \frac{\text{Number of colonies} \times \text{Dilution factor (if any)}}{\text{Plated volume (mL)}}$$

The amount of bacteria is expressed as colony-forming units per gram (CFU/g) in solid samples and per ml (CFU/ml) in liquid samples.

6.1.2. Direct microscopic counts

In this technique, cells are counted directly under a microscope using a counting chamber (a specialized slide), like Malassez counting chamber, Thomas counting chamber, and Petroff-Hausser counting chamber. The types of counting chambers differ in counting grids and the depths of the chambers. For example, the depth of the Malassez counting chamber is 0.2 mm, and the counting grid covers 2 mm x 2.5 mm = 5 mm² (Figure 68).

Figure 68. Malassez counting chamber.

The grid of a counting chamber is engraved into the surface of its base (Figure 69). The large rectangles have an area of 0.25 mm x 0.20 mm = 0.05 mm². Each of them is subdivided into 20 small squares (mini squares) with an area of 0.05 mm x 0.05 mm = 0.0025 mm². The volume of a large rectangle is 0.01 mm3 = 0.01 µl.

- **Calculation:** To calculate the number of cells/mL in the Malassez counting chamber (Determination of cell number in 10 rectangles), the following formula is used:

$$\text{Number of cells/mL} = \left(\frac{\text{Number of cell counted}}{\text{Total volume of counted rectangles}}\right) \times \text{Dilution factor (if any)}$$

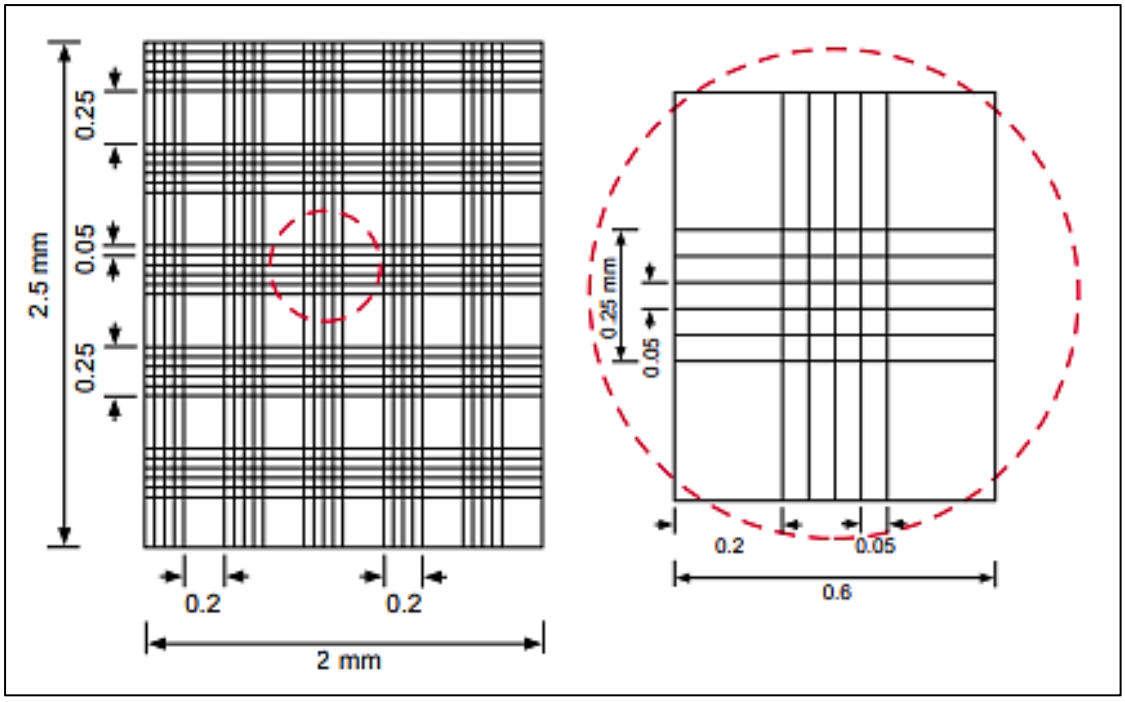


Figure 69. The counting grid in Malassez counting chamber.

6.1.3. Membrane filter method (Filtration)

In this method, bacteria are concentrated or trapped on a membrane filter by filtering a known volume of liquid sample (Figure 70). After filtration, the membrane filter is placed on an agar plate containing the suitable growth medium. The plates are then incubated at appropriate environmental conditions for colony growth. The number of bacterial colonies that grow on the membrane filter indicates the number of bacteria present in the volume of the sample filtered or passed through the membrane filter. The result of the bacterial population estimation is expressed as CFU/mL.

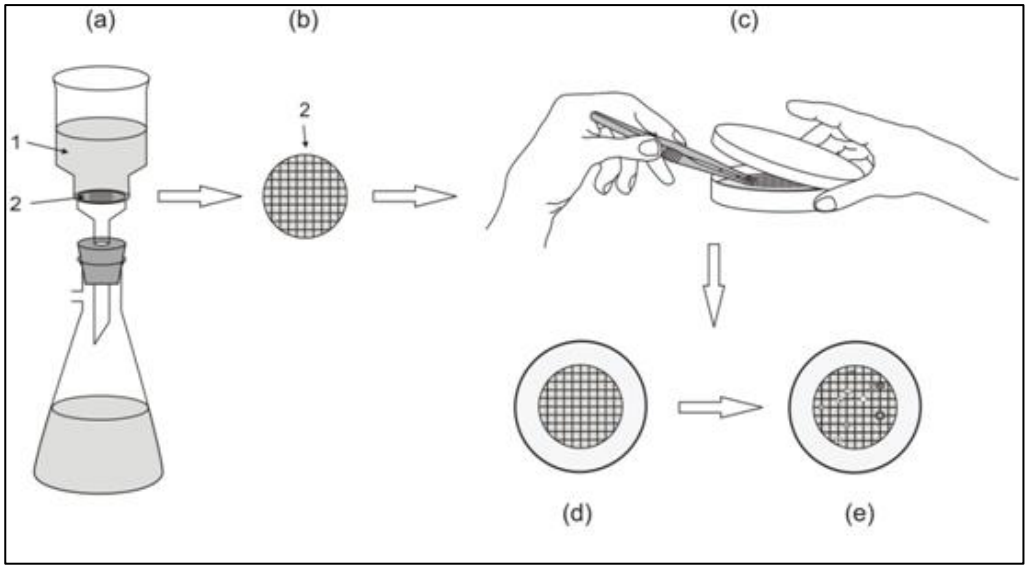


Figure 70. Bacterial count using the membrane filter method. **(a):** A known quantity of the test sample (1) is filtered through a membrane filter; **(b, c):** The membrane filter is placed onto a Petri plate using sterile forceps; **(d, e):** After incubation, colony forming unit (CFU) values on the surface of the filter can be counted.

6.2. Estimation of bacterial cell mass

6.2.1. Determination of dry weight

This technique is used to quantify bacteria by measuring their biomass rather than counting individual cells. Microbial biomass is centrifuged, washed, dried (at 105°C for 24 hours until constant weight is achieved), and weighed to obtain dry weight (to estimate cell concentration).

- **Calculation:** The quantification of bacteria (g/L) in the original sample is calculated using the following formula:

$$\text{Dry weight (g)} = \text{Weight of dried sample (g)} - (\text{Weight of empty container})$$

$$\text{Concentration (g/L)} = \frac{\text{Dry weight (g)}}{\text{Volume of sample (L)}}$$

6.2.2. Measurement of turbidity (Optical density)

A spectrophotometer measures the turbidity of a bacterial suspension. Light absorbance (optical density, OD) is measured (Figures 71a, 71b). OD is proportional to cell density. A calibration curve relating turbidity to cell count (determined by direct counting) allows estimation of cell numbers in unknown samples (Figure 71c).

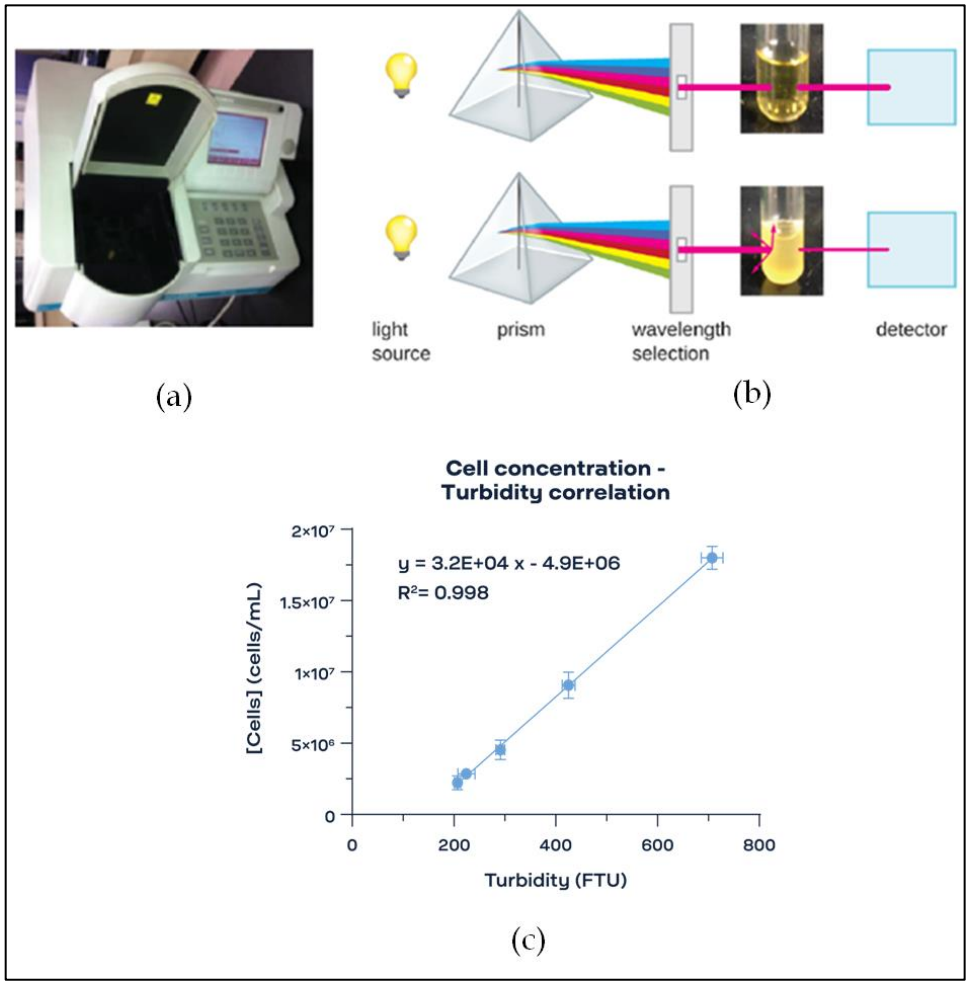


Figure 71. Measurement of turbidity. **(a):** Spectrophotometer; **(b):** Measurement of optical density; **(c):** Calibration curve.

Chapter V

Antimicrobial Agents

Chapter V. Antimicrobial Agents

Antimicrobial agents constitute a significant advancement in different fields, such as medicine, environment science, and food safety, providing essential resources for controlling microbial proliferation and preventing disease transmission. This chapter explores the fundamental principles, classification, mechanisms of action, and applications of these agents, offering essential knowledge for understanding microbial control in both clinical and environmental contexts.

1. Fundamental Concepts and Terminology

1.1. Sterilization

Sterilization is defined as the process of killing, complete elimination, or destruction of all forms of microbial life, including both vegetative cells and spores, from a medium, which may be a food environment, various solutions, or locations or surfaces with limited dimensions and sizes. Sterilization is carried out by various physical and chemical methods.

This absolute process ensures that no viable microorganisms remain, making it critical for surgical instruments, laboratory equipment, and certain pharmaceutical products

1.2. Disinfection

A temporary process that destroys or eliminates potentially pathogenic microorganisms. However, it does not eliminate all microbial contaminants, particularly bacterial spores. Disinfection is typically applied to inanimate surfaces and objects.

1.3. Disinfectants

Chemical antimicrobial agents can either inhibit the growth or destroy undesirable microorganisms on inert surfaces (tables, floors, medical-surgical equipment, etc.). However, they may not be able to eliminate all bacterial spores. Due to their high concentrations and potential toxicity, they are unsuitable for application to living tissues.

1.4. Antiseptics

Antimicrobial substances or agents designed for application to living tissues (intact skin, mucous membranes, wounds, etc.) that inhibit, destroy, or eliminate undesirable microorganisms without damaging the host tissue.

1.5. Decontamination

A temporary process that reduces microbial populations to safe levels according to public health standards, typically performed on contaminated equipment.

Additional precise terminology describes the action of antimicrobial agents against specific microorganisms:

- **Bactericidal substances:** destroy vegetative bacterial cells.
- **Bacteriostatic substances:** inhibit the growth of bacteria without killing them.

- **Fungicides:** used to destroy fungi, including their spores**.**
- **Sporicides:** used to destroy bacterial spores.
- **Virucides:** used to eliminate viruses.
- **Mycobacterial agents:** A chemical agent used to destroy mycobacteria.
- **Antibiotics:** represent a special category of antimicrobial agents - molecules capable of destroying bacteria (bactericidal effect) or inhibiting their growth (bacteriostatic effect) without significantly affecting the host eukaryotic cells.

2. Physical Antimicrobial Agents

2.1. Heat sterilization

With applications categorized into moist heat and dry heat techniques, heat remains one of the most reliable and extensively used methods for microbial control.

2.1.1. Moist heat sterilization

Moist heat sterilization is also referred to as steam sterilization. It employs steam (water vapor) instead of hot air for sterilization. This method is typically more efficient in destroying microorganisms than dry heat. This is because moist heat penetrates the walls of the microbial cell and spores more effectively, resulting in accelerated protein coagulation.

2.1.1.1. Sterilization by autoclave

It is widely recognized as one of the most effective, efficient, and rapid sterilization procedure. The principle of the method is based on the utilization of steam whose temperature is above the boiling point, which is achieved by increasing the applied pressure, typically at 121°C and 15 psi (1.5 bar) for 15-20 minutes. The combined effects of heat and steam (moist heat) destroy the protein structure of bacteria and other microbes, which ultimately results in their death.

In this method, an autoclave is used to carry out the sterilization procedure (Figure 72). It is a sturdy, double-walled metal container designed to withstand pressure, with components including an inner chamber and outer jacket, lid, pressure gauge, safety valve, temperature gauge, and steam generator.

Figure 72. Two different types of autoclaves.

- **Factors influencing autoclave sterilization time and temperature**

The effectiveness of autoclave sterilization depends on several factors, including:

- ✓ Expected microbial density of materials being sterilized and nature of contaminants.
- ✓ Composition of materials being sterilized (e.g., nutrient media containing carbohydrates and vitamins are usually sterilized at lower temperatures for longer periods).
- ✓ Container size of material being sterilized and their wall thickness.

- **What can and cannot be sterilized by autoclave?**

Several materials are suitable for autoclaving, such as water, culture media, surgical instruments, glassware, certain plastics, centrifuge tubes, pipette tips, hospital linens, and biomedical waste. However, acids, explosive materials, flammable substances, chlorine-based products, and radioactive materials should never be autoclaved.

2.1.1.2. Pasteurization

Louis Pasteur is known for inventing the process that bears his name, Pasteurization. It involves heating food and beverages (e.g., milk, juice, canned food) to temperatures below water's boiling point to destroy vegetative pathogenic microorganisms (mainly bacteria), followed by rapid cooling. This process eliminates pathogens and many spoilage microorganisms while preserving food quality. Pasteurization is not designed to eliminate all microorganisms in food.

Modern pasteurization methods include:

a) **Low-Temperature Long-Time Pasteurization (LTLT):** The food product is subjected to a temperature between 62 to 64°C for about 30 minutes, then immediately cooled to 5°C or below.

b) **High-Temperature Short-Time Pasteurization (HTST):** Useful for handling large quantities of milk. The liquid is heated to temperatures between 71.5 °C to 74 °C for approximately 15 to 30 seconds or between 74°C to 76°C for 15 to 20 seconds, followed by rapidly cooling to between 4°C and 5.5 °C using a continuous heat exchanger. HTST pasteurization targets harmful bacterial spores that are resistant (*Clostridium botulinum* spores). It should eliminate nearly all yeasts, mold, common spoilage, and harmful bacteria in milk.

c) **Ultra-high-temperature pasteurisation (UHT):** It is currently utilized to preserve milk. The milk is sterilized at 135°C for 2–5 seconds before packaging, then aseptically put into containers. Despite being left out of the refrigerator, UHT milk sometimes lasts six to nine months. UHT treatment is also anticipated to eliminate bacterial spores.

2.1.1.3. Tyndallization

Tyndallization is named after its inventor, John Tyndall. It is also known as intermittent sterilization. The method involves heating materials at 100°C for 30 minutes to 1 hour on three consecutive days, with 24-hour intervals between each heating. During these intervals, bacterial spores

that survived the initial heating germinate into vegetative cells, which are then destroyed in subsequent heating cycles. This ensures complete sterilization.

2.1.2. Dry heat sterilization

Dry heat sterilization causes the oxidation of microbial protoplasm. There are different methods of dry heat sterilization, such as hot air sterilization, direct flame, etc.

2.1.2.1. Hot air sterilization

It is carried out in a Pasteur oven (hot air oven) to sterilize glassware (such as Petri dishes, empty flasks, and test tubes), metal instruments (like needles, scissors, and scalpels), and ceramic tools (Figure 73). The process typically requires 160°C for 2 hours or 180°C for 90 minutes. Dry heat destroys microorganisms that contaminate sterile devices. Indeed, it rapidly dries out the cells and oxidizes their dry contents. It is less efficient than moist heat, requiring longer exposure times.

Figure 73. Pasteur oven (Hot air oven).

The following items cannot be sterilized using this method: flammable chemicals, liquid materials, culture media, and physiological solutions. Additionally, the oven should not include any plastic or glue that dissolves at high temperatures.

2.1.2.2. Flaming

It represents the simplest dry heat method, involving direct exposure to flame. This technique is commonly used for metal instruments like platinum wire loops, forceps, and scissors, typically using a Bunsen burner. Certain metal instruments, including spreaders and tweezers, may be sterilized by alcohol immersion followed by flaming.

2.2. Sterilization by radiation

2.2.1. Nonionizing radiations: ultra-violet rays

Ultraviolet (UV) radiation includes wavelengths from 100 nm to 400 nm in the electromagnetic spectrum. It is commonly used for sterilization and uses less energy than ionizing radiation, while still providing effective microbial control.

- **Mechanism of Action**

UV light exerts its antimicrobial effects primarily at wavelengths between 260-270 nm, corresponding to the maximum absorption by nucleic acids. At these wavelengths, UV radiation causes significant damage to microbial DNA through several mechanisms:

a) **Formation of Thymine Dimers**: UV light causes adjacent thymine molecules on the same DNA strand to form covalent bonds with each other, creating thymine dimers (Figure 74). This leads to the formation of mutations, which have the potential to kill microorganisms.

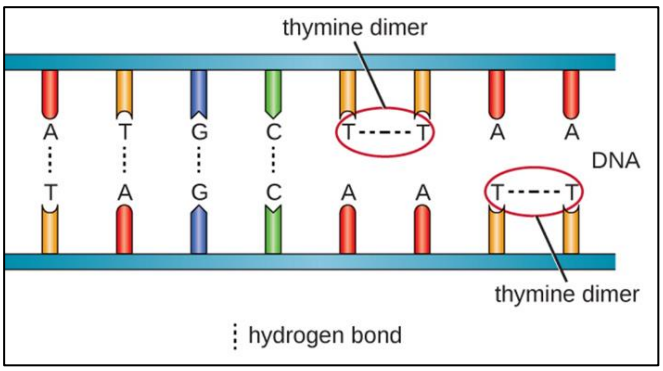


Figure 74. Thymine dimer formation caused by UV light.

b) **Disruption of DNA Replication:** When DNA polymerase encounters thymine dimers during replication, it cannot properly incorporate the appropriate complementary nucleotides. This leads to mutations that can be lethal to microorganisms.

c) **Free Radical Formation:** UV radiation can generate free radicals that cause additional damage to nucleic acids and other cellular components.

- **Limitations and practical considerations**

Despite its effectiveness, UV radiation has some important limitations:

✓ Poor Penetrating Power: UV light cannot penetrate surfaces, packaging materials, plastic, or glass. This restricts its use to surface sterilization only.

✓ Direct Exposure Requirement: Only microorganisms directly exposed to the UV light source are susceptible to destruction.

✓ Safety Concerns: UV light can damage human eyes, cause skin burns, and potentially induce mutations in skin cells, necessitating appropriate safety measures during its use.

- **Applications**

UV radiation finds application in various areas, including:

✓ Hospital operating rooms and sinks for reducing microbial contamination.

✓ Aseptic filling rooms in pharmaceutical manufacturing.

✓ Microbiological safety cabinets and hoods.

✓ Food and dairy processing equipment.

2.2.2. Ionizing radiations: X-rays and Gamma rays

Ionizing radiation, including X-rays and gamma rays, is an effective way to sterilize heat-sensitive and packaged materials. X-rays and gamma rays can both be used to sterilize a variety of packaged materials since they have much more energy and penetrating power than ultraviolet radiation. It can effectively sterilize a variety of packaged materials as it easily penetrates paper, plastic, thin sheets of wood and metal. Its antimicrobial action occurs through both direct and indirect mechanisms:

1. **Direct Effects**: Ionizing radiation directly damages DNA and proteins within microbial cells.
2. **Indirect Effects**: Radiation ionizes water and other molecules to form radicals (molecular fragments with unpaired electrons), which subsequently damage cellular components.

- **Applications of ionizing radiation**

Ionizing radiation is particularly used for sterilizing:

- ✓ Materials that cannot withstand autoclaving, such as plastic Petri dishes, disposable plastic inoculating loops).
- ✓ Medical supplies (gloves, intravenous tubing, and other latex and plastic items), and pharmaceutical drugs.
- ✓ Heat-sensitive materials used clinically, including biological tissues for transplantation.

- **X-rays as sterilizing agents**

X-rays are more effective against microorganisms than ultraviolet radiation due to their ionizing properties and superior penetrating ability. They can destroy microbial cells by altering genetic material and causing mutations, or by killing cells outright when applied in sufficient concentrations.

Despite these advantages, X-ray sterilization faces practical limitations due to its high production costs. In addition, it has omnidirectional radiation which spreads from the source. However, X-rays maintain value in research settings, particularly for producing microbial mutants for scientific investigation.

- **Gamma rays as sterilizing agents**

Gamma rays share similarities with X-rays in their lethal effects on microorganisms but have shorter wavelengths. They are highly effective for internal sterilization of thick or large objects due to their ability to penetrate them. Gamma radiation is particularly beneficial for sterilizing several objects, such as canned foods, antibiotics, vitamins, and single-use plastic items like Petri dishes and membrane syringe filters.

2.3. Sterilization by filtration

It is unique among sterilization techniques in that it physically removes, rather than destroys microorganisms from solutions while preserving the chemical integrity of heat-sensitive materials.

Sterilization by filtration, often referred to as cold sterilization, involves the physical separation of microorganisms from liquids by passing the solution through a membrane filter with pores small enough to prevent the passage of microbes but large enough to allow the organism-free liquid to pass through. This method is particularly valuable for sterilizing solutions that would be damaged by heat or chemical treatments.

The effectiveness of filtration sterilization depends primarily on the pore size of the filter membrane. Standard microbiological filters used for bacterial removal typically have a pore size of 0.2 µm. However, filters with smaller pore sizes are available for more specialized applications. As a general principle, the smaller the pore size, the more effective the filter is at removing microorganisms and particulates from the solution.

Several filtration systems are commonly used in laboratory and industrial settings:

- **Vacuum Filtration Systems**

The Buchner funnel vacuum device represents one of the most widely used filtration setups in laboratory settings (Figure 75). This system consists of a Buchner funnel that holds the filter membrane, a flask to collect the filtered liquid, a vacuum source that creates negative pressure to draw the liquid through the filter, a rubber adapter or a clamp that connects the funnel to the flask and maintains an airtight seal. These systems are particularly useful for filtering larger volumes of liquid efficiently. The vacuum reduces filtration time by actively pulling the liquid through the membrane rather than relying solely on gravity.

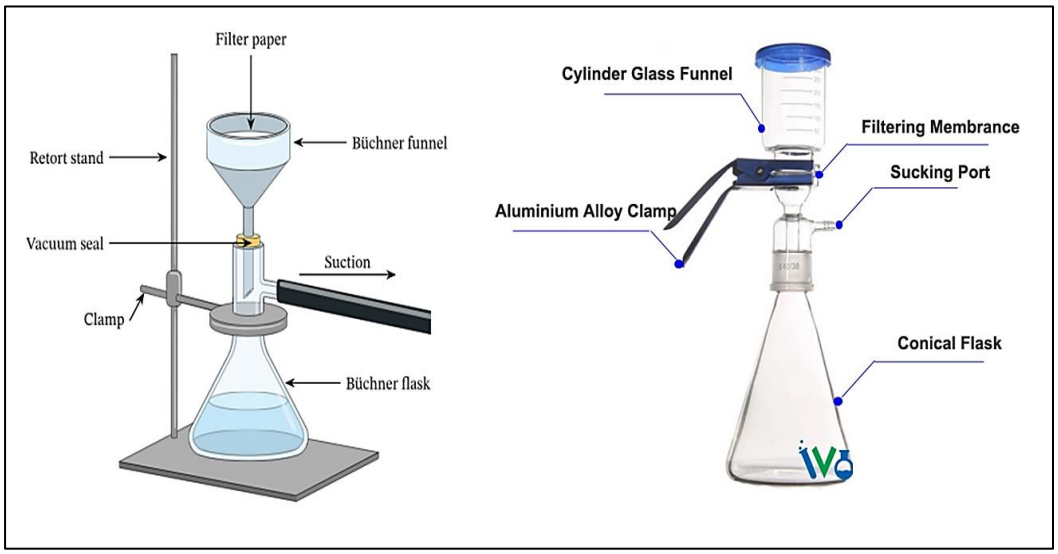


Figure 75. Different vacuum filtering apparatus.

- **Sterile syringe filters**

Sterile syringe filters provide a convenient and disposable option for smaller volumes (Figure 76). They consist of a range of available pore sizes (0.22 µm, 0.45 µm, etc.) for different filtration needs. Syringe filters are particularly useful in research laboratories where small volumes of sterile solutions

are frequently needed. They offer the advantages of being single-use (eliminating cross-contamination concerns) and requiring minimal setup.

Figure 76. Sterilization by filtration using sterile syringe filters.

Microbiological membrane filters provide a useful way of sterilizing heat-sensitive solutions, such as vaccines containing protein antigens, antibiotic solutions, animal sera used in cell culture applications, enzyme solutions, and vitamin solutions with heat-sensitive components.

3. Chemical Antimicrobial Agents

3.1. Disinfectants and antiseptics

Chemical antimicrobial agents can be classified into two main categories: disinfectants which destroy or inhibit the growth of undesirable microorganisms on inert surfaces, and antiseptics which destroy or inhibit the growth of undesirable microorganisms without damaging the host tissue.

3.1.1. Factors influencing antimicrobial action

Different factors affect the efficacy of disinfectants and antiseptics, including:

- ✓ **Concentration:** Higher concentrations generally provide greater antimicrobial activity, though optimal concentrations vary by agent.
- ✓ **Temperature**: In general, disinfection or decontamination takes longer at lower temperatures.
- ✓ **Types of microorganisms:** Endospore-forming bacteria (such as *Bacillus*, and *Clostridium*), and acid-fast bacteria (like *Mycobacterium tuberculosis*) are particularly harder to eliminate.
- ✓ **Microbial load**: The greater the number of microorganisms present, the more difficult disinfection becomes. Best results are achieved when initial microbial numbers are low and surfaces to be disinfected are clean and free of interfering substances

3.1.2. Mode of action

Disinfectants and antiseptics typically act through two common modes of action:

- ✓ **Membrane damage:** They damage lipids and/or proteins in the semipermeable cytoplasmic membrane of microorganisms, causing leakage of cellular materials essential for survival.

- ✓ **Protein denaturation:** They denature microbial enzymes and other proteins by disrupting hydrogen and disulfide bonds, which alters the protein three-dimensional structure and blocks metabolism.

3.1.3. Common chemical antimicrobial agents

3.1.3.1. Alcohols

Ethyl alcohol (ethanol) and isopropyl alcohol (isopropanol) are widely utilized as antiseptics and disinfectants. They are bactericidal and fungicidal, but not sporicidal agents. Solutions of 70% ethanol or isopropanol are most effective in eliminating vegetative bacteria, enveloped viruses, and fungi (solutions below 50% are less effective, while those above 90% evaporate too rapidly). However, they are usually ineffective against endospores and non-enveloped viruses.

Alcohols function by denaturing and coagulating proteins, destroying cell membranes, and disrupting viral envelopes.

3.1.3.2. Aldehydes

Aldehyde disinfectants include formaldehyde, glutaraldehyde, and ortho-phthalaldehyde (OPA). They denature microbial proteins and damage nucleic acids, irreversibly inhibiting microbial enzyme activity. They are highly effective, broad-spectrum disinfectants that act slowly but effectively against bacteria and enveloped viruses. They are less effective against non-enveloped viruses, bacterial spores, and acid-fast bacteria.

- **Formaldehyde**

Formaldehyde exists as a gas or liquid. Formalin (37% aqueous solution of formaldehyde gas) is extremely effective and destroys most forms of microbial life. It is utilized in embalming, preserving biological specimens, and vaccine preparation. At 4-10% concentration, serves as a moderate to high-level disinfectant. At high concentrations, formaldehyde can destroy all microorganisms, including spores (sterilization). Use is limited due to carcinogenicity concerns.

- **Glutaraldehyde**

Glutaraldehyde is widely used for high-level disinfection of medical equipment (e.g., endoscopes). At a 2% concentration, it is effective and utilized for high-level disinfection. It can also act as a sterilant with prolonged contact times. It may be less toxic than formaldehyde but can irritate. Glutaraldehyde is considered non-corrosive and does not damage rubber and plastics, but may be mildly corrosive to metals. It is more effective in the presence of organic matter, soaps, and hard water than formaldehyde.

- **Ortho-phthalaldehyde (OPA)**

It is a high-level disinfectant, such as Cidex, which destroys vegetative bacteria in 10-30 minutes and endospores in about 4 hours. It is used for reprocessing reusable heat-sensitive semi-critical medical devices.

3.1.3.3. Halogens

Halogen-based compounds include chlorine (e.g., sodium hypochlorite/bleach, chlorine dioxide) and iodine-containing agents. Their antimicrobial effect stems from their electronegative nature, which denatures proteins, enzymes, and disrupts lipid membranes.

- **Chlorine**

Chlorine gas is commonly added to water to form hypochlorite (active ingredient in bleach). Hypochlorite denatures microbial enzymes, destroys bacteria and inactivates viruses. It is used in drinking water, swimming pools, and sewage treatment. It is also available as liquid sodium hypochlorite (household bleach) or solid calcium hypochlorite (swimming pool chemical). As an antiseptic, chlorine is used in formulations like Dakin solution (sodium hypochlorite and potassium permanganate).

The mechanism of action of chlorine includes the oxidation of sulfhydryl enzymes and amino acids, chlorination of amino acid rings, loss of intracellular contents, decreased nutrient uptake, inhibition of protein synthesis, decreased oxygen uptake, oxidation of respiratory components, decreased ATP production, DNA breaks and depressed DNA synthesis.

- **Iodine and Iodophors**

Iodine denatures microbial proteins. Aqueous iodine solutions (2% iodine and 2.4% sodium iodide) are frequently used as topical antiseptics. Iodophors are a combination of iodine and a solubilizing agent that facilitates the slow release of iodine in solution. Iodophors are less irritating than iodine. They are broad-spectrum disinfectants and are generally effective against vegetative bacteria, *M. tuberculosis*, fungi, some viruses, and some endospores.

Iodophors function by penetrating the cell walls and membranes of microorganisms, interfering with DNA synthesis, and binding to proteins. The most common iodophor is Povidone-iodine (Betadine), which is an iodine bound to polyvinylpyrrolidone.

3.1.3.4. Hydrogen Peroxide (Oxygenated Water)

It is active against a wide range of microorganisms, including bacteria, yeasts, fungi, viruses, and spores. It has good germicidal activity: bactericidal, virucidal, sporicidal, and fungicidal properties. It attacks membrane lipids, DNA, and other essential cell components.

3.1.3.5. Phenol and Its Derivatives

Phenol was the first widely used antiseptic. It is not commonly used today due to toxicity, irritation, corrosive properties, and odor. Phenol is a toxic compound whose vapors are corrosive to the skin, eyes, and respiratory tract.

The mode of action of phenol includes altering membrane permeability, disrupting cells, denaturing proteins, inactivating enzymes, and causing loss of amino acids from cells. Phenols are

slowly effective against spores (non-sporicidal). The are effective against a wide range of microorganisms, including bacteria, fungi, and viruses.

3.1.3.6. Heavy Metals

Heavy metals, such as mercury, silver, and copper denature proteins. Mercury compounds are bacteriostatic but ineffective against endospores. Silver nitrate (1%) is utilized to prevent gonococcal ophthalmia in newborns. Copper sulfate is used against fungal plant diseases and as an algicide to control algal growth in swimming pools and fish tanks. Zinc compounds (zinc chloride, zinc oxide) are also commercially used. Zinc chloride is commonly found in mouthwashes. Mercury is also an example of a heavy metal used for many years to control microbial growth. Various forms of mercury bind to sulfur-containing amino acids in proteins, inhibiting their functions. The use of such compounds has diminished due to mercury's toxicity.

3.1.3.7. Quaternary Ammonium Compounds (QACs)

Quaternary Ammonium Compounds (QACs) are widely used as disinfectants. They are non-corrosive with low toxicity. They are cationic detergents (like benzalkonium chloride).

The mode of action of QACs includes inactivating energy-producing enzymes, denaturing of cellular proteins, and disrupting of cell membranes. They are generally bactericidal, virucidal (effective against enveloped viruses), not sporicidal, and have limited fungicidal action. They are used for environmental sanitation of noncritical surfaces and disinfection of medical equipment that contacts intact skin.

3.1.3.8. Soaps and detergents

They are used as surface-active agents, wetting agents, and emulsifiers. Detergents may be anionic or cationic. Anionic detergents (like laundry powders) mechanically remove microorganisms but have limited microbicidal activity.

4. Chemotherapeutic Agents: Antibiotics

Chemotherapeutic agents are chemicals used internally to kill or inhibit the growth of microorganisms within host tissues. The term "chemotherapy" was introduced by Ehrlich in 1904 to describe the use of synthetic chemicals to destroy infectious agents. Chemotherapy is a drug treatment with highly selective toxicity toward bacteria and other pathologic microorganisms, parasites, and tumour cells while minimizing host toxicity. Chemotherapeutic agents can be divided into:

- Antimicrobial agents: Including antibacterial, antifungal, and antiviral agents.
- Antiparasitic agents: Including anthelmintics and antiprotozoal agents.
- Antineoplastic agents: Anticancer.

4.1. Definition of antibiotics

Antibiotics are chemical compounds derived from diverse microorganisms (bacteria, fungi, actinomycetes) that can destroy (bactericidal) or inhibit (bacteriostatic) the growth of other

microorganisms. They are effective at very low concentrations and represent the most important type of antibacterial agent for combating bacterial infections.

4.2. General characteristics of antibiotics

Two key characteristics define antibiotics:

a) **Selective toxicity**: The ability to kill or inhibit microorganism growth without harming host cells — a crucial aspect of antimicrobial therapy. In most instances, this selective toxicity is relative rather than absolute.

b) **Antimicrobial spectrum**: antibiotics can be:

- **Narrow-spectrum**: Active against a limited group of microorganisms, often affecting only gram-positive or gram-negative bacteria but not both. For example, isoniazid is active only against Mycobacteria.
- **Broad-spectrum**: Effective against a wide range of microbial species, including both gram-positive and gram-negative bacteria. Examples include tetracyclines and chloramphenicol.

4.3. Classification of antibiotics

Antibiotics can be classified based on their chemical nature and mechanism of action.

4.3.1. Classification based on chemical structure

4.3.1.1. Beta-Lactams

Beta-lactams (β-lactams) contain a 3-carbon and 1-nitrogen ring that is highly reactive (Figure 77). The most prominent members include penicillins, cephalosporins, monobactam, and carbapenems.

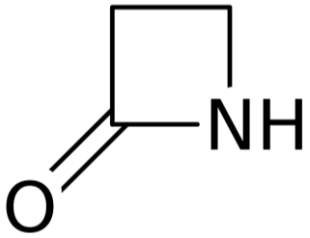


Figure 77. Chemical structure of a beta-lactam ring.

a) Penicillins

Penicillin was the first antibiotic discovered by Alexander Fleming in 1929. Penicillins are β-lactam compounds with a nucleus of 6-aminopenicillanic acid ring and other ring-side chains (Figure 78). Members of penicillin group include: penicillin G, penicillin V, oxacillin, ampicillin, methicillin, nafcillin, amoxicillin, carbenicillin, piperacillin, mezlocillin, and ticarcillin.

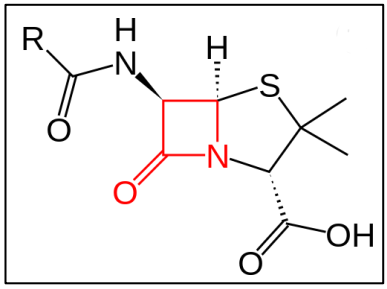


Figure 78. Chemical structure of a 6-aminopenicillanic acid ring.

Penicillin G was originally isolated from the fungus *Penicillium notatum* (*P. chrysogenum*). It has a narrow spectrum, effective against Gram-positive bacteria (Streptococci) and some Gram-negative bacteria like *Treponema pallidum* and meningococci. Some penicillins (e.g., Ampicillin, Carbenicillin, Amoxicillin) have been developed semi-synthetically with different side-chains. Augmentin is a drug comprising amoxicillin (antibiotic) and clavulanic acid, a non-antibiotic compound that inhibits beta-lactamase enzymes, extending its effectiveness against penicillinase-producing bacteria.

b) Cephalosporins

Cephalosporins refer to a variety of semisynthetic antibiotics derived from Cephalosporin C, isolated in 1945 from the fungus *Cephalosporium acremonium*. Cephalosporin C is similar to penicillin in structure and mode of action. Cephalosporins contain 7-aminocephalosporanic acid nucleus and side chain containing 3,6-dihydro-2H-1,3-thiazane rings (Figure 79).

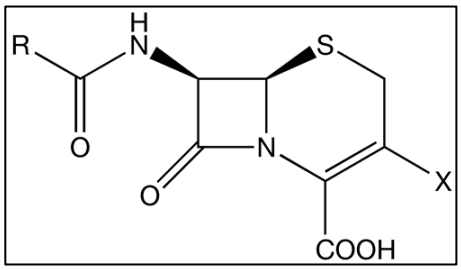


Figure 79. Chemical structure of 7-aminocephalosporanic acid nucleus.

Cephalosporins are classified into generations:

- **First-generation:** Highest activity against gram-positive organisms, lowest against gram-negative.
- **Second-generation:** More active against gram-negative bacteria, less active against gram-positive.
- **Third-generation:** Greater spectrum against gram-negative bacteria and more resistant to gram-negative β-lactamase enzymes.
- **Fourth-generation:** Improved gram-positive spectrum while retaining expanded gram-negative activity.

c) Monobactams

Monobactams differ from other β-lactams by their monocyclic ring structure (Figure 80). Aztreonam, a synthetic monocyclic β-lactam, is the prototype drug in this group. The core structure

was originally isolated from the Gram-negative bacteria *Chromobacterium violaceum*. It is active only against aerobic gram-negative bacteria (e.g., *Pseudomonas*, *Neisseria*). It has a significantly narrower spectrum than other β-lactams.

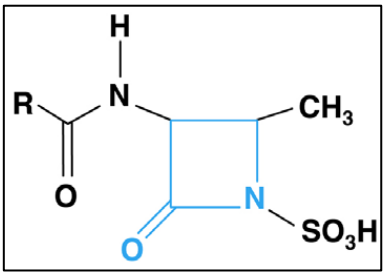


Figure 80. Chemical structure of a monobactam nucleus.

d) Carbapenems

Carbapenems are a class of β-lactam natural products produced by soil-dwelling streptomycetes. They have been developed for clinical use in difficult-to-treat infections. They resist the hydrolytic action of beta-lactamase enzymes. Among the several known β-lactams, carbapenems have the broadest spectrum of activity and greatest potency against Gram-positive and Gram-negative bacteria. Examples include Imipenem, Meropenem, and Ertapenem.

4.3.2. Macrolides

A class of antibiotics that are produced by bacteria belonging to the order Actinomycetales. They have a wider spectrum of antibiotic activity than penicillins and are often administered to patients with penicillin allergies. Macrolides either kill or inhibit microorganisms by effectively inhibiting bacterial protein synthesis. Examples include Erythromycin, Clarithromycin, and Azithromycin.

4.3.3. Tetracyclines

The first member of this class is chlorotetracycline (Aureomycin), which was discovered in 1945 from a soil bacterium of the genus *Streptomyces* by Benjamin Duggar. Tetracyclines contain four benzene rings (Figure 81).

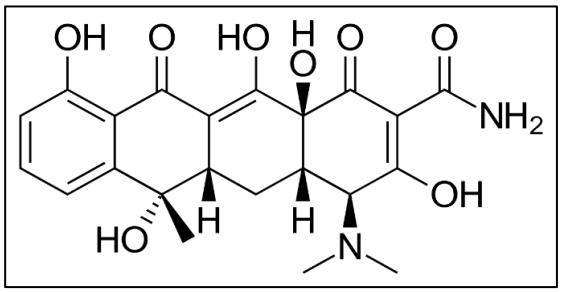


Figure 81. The fundamental structure of tetracyclines.

Tetracyclines are classified into generations:

- **First generation (biosynthesis):** Tetracycline, Chlortetracycline, Oxytetracycline, Demeclocycline.

- **Second generation (semi-synthesis):** Doxycycline, Lymecycline, Meclocycline, Minocycline, Rolitetracycline.
- **Third generation (total synthesis):** Tigecycline.

4.3.4. Quinolones

This class of antibiotics was first discovered as nalidixic acid during the search for antimalarial drugs. The structure of quinolones generally has two rings (Figure 82), while newer generations have an additional ring structure. It enables them to extend their spectrum of antibacterial activity to some bacteria, particularly anaerobic bacteria.

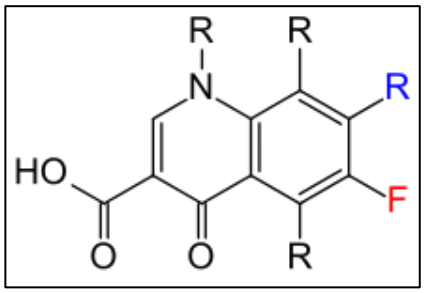


Figure 82. The fundamental structure of quinolones.

Quinolones are classified into generations:

- **First-generation:** Nalidixic acid, Cinoxacin, Norfloxacin, Lomefloxacin (active against Gram-negative bacteria but not *Pseudomonas*).
- **Second-generation:** Enoxacin, Ofloxacin, Ciprofloxacin, Levofloxacin (active against Gram-negative bacteria including *Pseudomonas*, and some Gram-positive bacteria).
- **Third-generation:** Moxifloxacin, Gatifloxacin, Sparfloxacin (expanded Gram-positive coverage including penicillin-resistant *S. pneumoniae*).

4.3.5. Aminoglycosides

Aminoglycosides are natural or semisynthetic antibiotics derived from Actinomycetes. They are compounds of usually 3-amino sugars connected by glycosidic bonds. They are potent with a broad spectrum of antibacterial activity. They inhibit protein synthesis in bacteria by binding to ribosomal subunits. They are effective against aerobic Gram-negative rods and certain Gram-positive bacteria. Streptomycin was the first discovered (1943) and used against *M. tuberculosis*. Other examples include Neomycin, Kanamycin, Gentamicin, Tobramycin, and Amikacin.

4.3.6. Sulfonamides

Sulfonamides are synthetic antimicrobial agents containing the sulfonamide chemical group. They inhibit the growth of various Gram-positive and Gram-negative bacteria, including *Nocardia*, *E. coli*, *Klebsiella*, *Salmonella*, *Shigella*, *Enterobacter*, *Chlamydia trachomatis*, as well as some protozoa. Sulfonamides are used in treating various infections, such as urinary tract infections.

4.3.7. Glycopeptides

Glycopeptides are naturally obtained from Actinomycetes soil bacteria. They are naturally composed of a heptapeptide skeleton (a cyclic peptide of 7 amino acids) bound to 2 sugars. They inhibit bacterial growth by interfering with cell wall biosynthesis. Examples include Vancomycin (produced by *Streptomyces orientalis*) and Teicoplanin. However, semi-synthetic derivatives (Dalbavancin and Oritavancin) have improved activity and pharmacokinetic properties.

4.3.8. Oxazolidinones

Oxazolidinones are a group of synthetic antibiotics approved relatively recently. Linezolid, the first member, was approved for clinical application in 2000 by the Food and Drug Administration (FDA). It is used for treating respiratory tract and skin infections caused by Gram-positive bacterial pathogens. Oxazolidinones have a broad spectrum of activity against Gram-positive bacteria, including vancomycin-resistant enterococci, penicillin-resistant pneumococci, and anaerobes.

4.3.2. Classification based on mechanism of action

4.3.2.1. Inhibition of cell wall synthesis

Antibiotics that inhibit cell wall synthesis target peptidoglycan, an essential component of bacterial cell walls responsible for the shape, mechanical strength, and integrity of bacterial cells. Since mammalian cells lack cell walls, these antibiotics have excellent selective toxicity.

Peptidoglycan biosynthesis can be divided into three stages: precursor production in the cytoplasm, assembly on lipid carriers in the cell membrane and transport from the cytoplasm to the cell wall and integration into the existing peptidoglycan network in the cell wall. Two key reactions in the third stage are primary targets for antibiotics: transglycosylation (formation of linear, non-cross-linked glycan chains) and cross-linking (transpeptidation reactions connecting peptide substituents).

Glycopeptide antibiotics like vancomycin bind tightly to the terminal D-alanyl-D-alanine on peptidoglycan precursors, forming five hydrogen bonds with the target. This binding prevents interaction with Penicillin Binding Proteins (PBPs) and blocks incorporation of new subunits into the growing peptidoglycan (Figure 83).

β-lactams antibiotics (penicillins, cephalosporins, carbapenems, and monobactams) mimic the structure of D-alanyl-D-alanine, irreversibly binding to and inactivating transpeptidase enzymes (PBPs). This prevents cross-linking of peptidoglycan strands, leading to the weakening of the cell wall and eventual bacterial lysis. Other cell wall synthesis inhibitors include bacitracin, which inhibits the transport of peptidoglycan subunits by inactivating the phospholipid carrier, and cycloserine, which inhibits D-alanyl-D-alanine synthesis inside the cell (Figure 83).

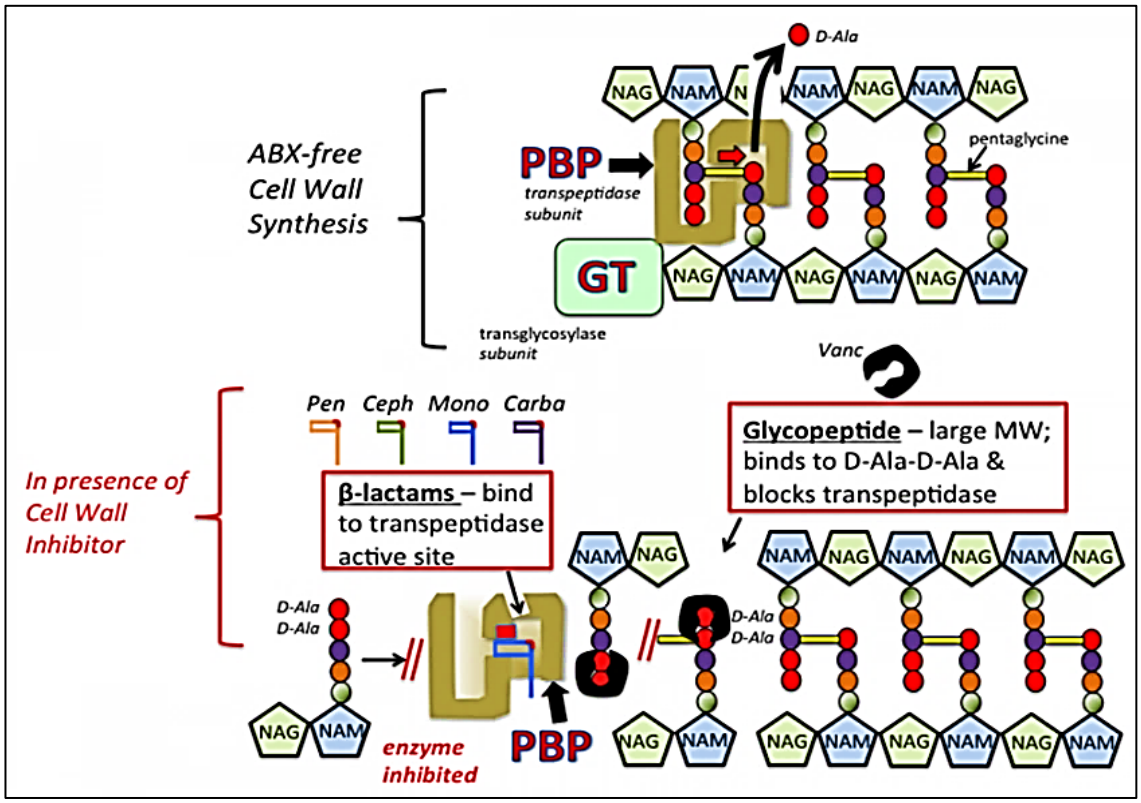


Figure 83. Inhibition of cell wall synthesis by β-lactams and glycopeptides.

4.3.2.2. Inhibition of protein synthesis

a) Aminoglycosides (30S Inhibitors)

Aminoglycosides are broad-spectrum bactericidal antibiotics. They bind to the 30S ribosomal subunit of the bacterial initiation complex. They cause the misreading of messenger RNA (mRNA) and the premature termination of translation. They require oxygen-dependent active transport for cellular uptake. Examples include Streptomycin, Gentamicin, Tobramycin, and Amikacin.

b) Tetracyclines (30S Inhibitors)

Tetracyclines are bacteriostatic antibiotics. Like aminoglycosides, they bind to the 30S ribosomal subunit. They reversibly inhibit tRNA binding to the acceptor site (A site), hinder polypeptide chain growth.

c) Chloramphenicol (50S Inhibitors)

Chloramphenicol is a broad-spectrum antibiotic which binds to the 50S ribosomal subunit and prevents binding of tRNA to the A site. It has both bacteriostatic and bactericidal properties.

d) Macrolides (50S Inhibitors)

Macrolides affect the early stages of protein synthesis (translocation). They target the peptidyl transferase center of the 23S rRNA in the 50S subunit. They cause premature detachment of incomplete peptide chains. Examples include erythromycin and azithromycin (used for respiratory and Chlamydia infections).

e) Oxazolidinones (50S Inhibitors)

They inhibit the formation of the initiation complex on the 50S ribosomal subunit. They are effective against vancomycin-resistant Enterococci and penicillin-resistant *S. pneumoniae*.

4.3.2.3. Inhibition of nucleic acid synthesis

a) Sulfonamides (folic acid metabolism inhibitors)

Sulfonamides inhibit folic acid synthesis by competitively inhibiting p-aminobenzoic acid (PABA). They competitively inhibit the enzyme dihydropteroate synthase and block the synthesis of tetrahydrofolic acid, required for nucleic acid synthesis. Sulfonamides do not affect human cells because mammals rely on dietary folic acid

b) Quinolones (DNA replication inhibitors)

Quinolones are synthetic, orally active bactericidal antibiotics that inhibit DNA gyrase and topoisomerase IV. In Gram-negative bacteria, they primarily target DNA gyrase, while in Gram-positive bacteria, they primarily target topoisomerase IV. They prevent DNA replication and transcription.

c) Rifamycins (DNA transcription inhibitors)

Rifamycins selectively bind to the β subunit of bacterial RNA polymerase, preventing the initiation of RNA transcription. Rifampin is specifically used against the pathogen *M. tuberculosis*.

4.3.2.4. Disruption of Cytoplasmic Membrane

Polymyxins antibiotics act on the outer membrane of Gram-negative bacteria. They bind to negatively charged phosphate groups on lipopolysaccharides, disrupting membrane integrity and causing leakage of cytoplasmic contents. They are useful against many Gram-negative rods and carbapenemase-producing Enterobacteriaceae. The antibiotic colistin (Polymyxin E) is used for serious Gram-negative infections.

4.4. Mechanisms of antimicrobial resistance

4.4.1. Definition of antibiotic resistance

Antibiotic resistance is defined as the ability of bacteria or other microorganisms to survive and reproduce in the presence of antibiotic doses that were previously thought to be effective against them.

4.4.2. Origins of Resistance

Antimicrobial resistance can be either intrinsic (naturally occurring in a bacterial species) or acquired through genetic changes. Intrinsic resistance is a trait shared universally within a bacterial species, independent of previous antibiotic exposure and not related to horizontal gene transfer. Acquired resistance occurs through several mechanisms:

- ✓ Horizontal gene transfer (transformation, transposition, conjugation).
- ✓ Mutations in chromosomal DNA.
- ✓ Acquisition of plasmids carrying resistance genes.

4.4.3. Mechanisms of antibiotic resistance

Bacteria have numerous mechanisms to evade the effects of antibiotics. These mechanisms can be categorized into four primary strategies:

4.4.3.1. Reduced Membrane Permeability

This mechanism represents a common way for bacteria to interfere with antibiotic efficacy. Key aspects include:

- Porin alteration and mutation effects: Gram-negative bacteria possess an outer cell membrane through which drugs must pass via porins (channel proteins). Gene mutations can alter the electrical charge or physical structure of these porins, making it more difficult for antibiotics to enter the cell.
- Multidrug resistance: This mechanism can confer resistance to multiple drug classes simultaneously.
- Intrinsic resistance: Some gram-negative bacteria possess innate resistance to large antibiotics like vancomycin, which are too large to pass through the porins even before mutations occur.

4.4.3.2. Efflux Pumps

Efflux pumps represent another significant mechanism of antibiotic resistance. These membrane proteins actively export antibiotics out of the cell before they can reach their target sites. Unlike porins, which are located in the outer membrane, efflux pumps are present in the cytoplasmic membrane. Most of them are multidrug transporters capable of pumping out a wide range of unrelated antibiotics, including macrolides, tetracyclines, and fluoroquinolones. Thus, efflux pumps significantly contribute to the development of multidrug-resistant organisms.

4.4.3.3. Modification of target molecule or site

Many antibiotics act by binding to specific molecular targets within bacteria. Resistance can develop when microorganisms modify the structure of the target molecule, preventing antibiotic binding while maintaining normal cellular function. Since antibiotic-target interactions are generally quite specific, even minor alterations of the target molecule can significantly affect antibiotic binding. These target site changes often result from spontaneous mutations in bacterial chromosomal genes. Examples include:

- ✓ Ribosomal alterations: Changes in the 30S or 50S ribosomal subunits confer resistance to protein synthesis inhibitors like macrolides, tetracyclines, chloramphenicol, and aminoglycosides.
- ✓ PBP modifications: Alterations in penicillin-binding proteins (PBPs) represent a favored resistance mechanism in Gram-positive bacteria against β-lactam antibiotics. This mechanism explains the resistance of *Enterococcus faecium* to ampicillin and *S. pneumoniae* to penicillin.
- ✓ Cell wall precursor modifications: In vancomycin resistance, D-alanyl-D-alanine residues in peptidoglycan precursors are changed to D-alanyl-lactate, preventing glycopeptide binding. This mechanism is observed in vancomycin-resistant *E. faecium* and *E. faecalis*.

4.4.3.4. Enzymatic inactivation of antibiotics

Both Gram-positive and Gram-negative bacteria can synthesize enzymes that degrade or modify antibiotics. This enzymatic inactivation represents one of the most important resistance mechanisms. The classic example is β-lactamases, which hydrolyze the β-lactam ring of penicillins and cephalosporins (Figure 84). These enzymes open the β-lactam ring structure, preventing the antibiotic from binding to its target PBPs. β-lactamases are widespread and represent the most common resistance mechanism used by Gram-negative bacteria against β-lactam antibiotics.

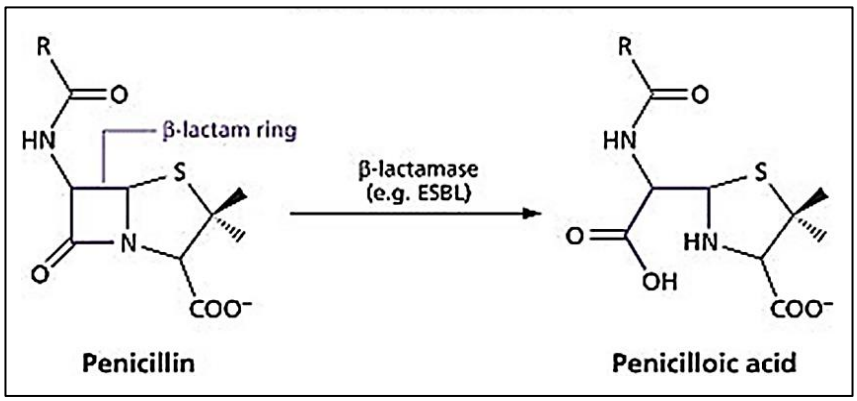


Figure 84. Enzymatic inactivation of penicillin by β-lactamases.

4.4.4. Antibiotic susceptibility testing

4.4.4.1. Disc Diffusion Test (Kirby-Bauer susceptibility test)

The disc diffusion test represents one of the easiest and efficient methods for evaluating antibiotic sensitivity of bacterial isolates. This test is used to determine the sensitivity or resistance of pathogenic aerobic and facultative anaerobic bacteria to various antibiotics, providing valuable information to clinicians treating patients with bacterial infections.

- **Methodology**

The testes bacteria is swabbed onto a Mueller-Hinton (MH) agar plate. Then, small filter paper discs (6 mm in diameter) pre-impregnated with known quantities of antibiotics are placed on the agar surface. The plates are incubated for 18-24 hours at 37°C, during which the antibiotic diffuses from the disc into the surrounding agar, creating a concentration gradient. The antibiotic concentration is highest adjacent to the disc and decreases logarithmically as the distance from the disc increases.

After incubation, results are interpreted as follows: if the bacterial strain is sensitive to the antibiotic, a clear zone of inhibition (where no bacterial growth occurs) appears around the disc (Figure 85). The diameter of this inhibition zone is measured in millimeters (mm) and compared against established breakpoints. Based on these measurements, bacteria are classified as susceptible (S), intermediate (I), or resistant (R).

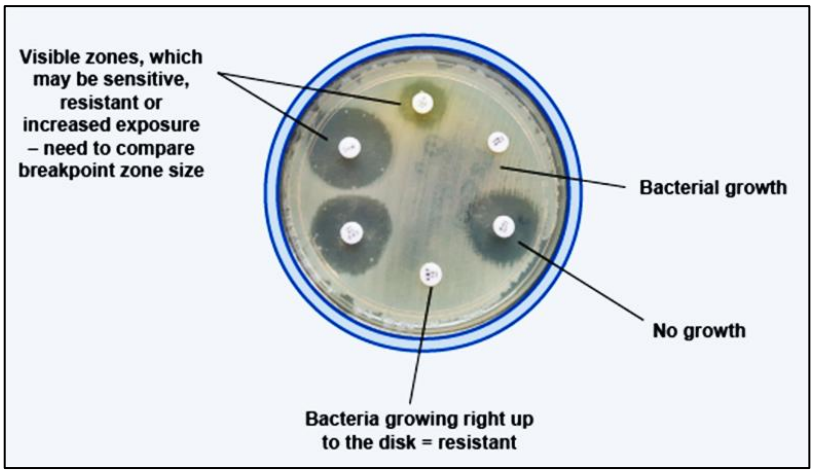


Figure 85. Kirby-Bauer susceptibility test.

- **Factors affecting zone diameter**

The diameter of the inhibition zone depends on several factors, including the quantity of antibiotic within the disc, degree of bacterial susceptibility to the antibiotic, physico-chemical properties of the antibiotic (solubility, molecular weight), agar plate depth (in mm), and bacterial concentration in the inoculum.

4.4.4.2. Broth dilution method

The broth dilution method provides quantitative results by determining the minimum inhibitory concentration (MIC) of antibiotics against bacterial isolates.

- **Methodology**

This technique involves creating a series of doubling dilutions of the antibiotic in a liquid culture medium, yielding a range of concentrations in either test tubes (macrodilution) or microtiter trays (microdilution). The bacterial strain is inoculated into each antibiotic concentration, and the inoculated dilutions are incubated for 18-24 hours. After incubation, MIC is determined by observing visible turbidity in the broth. The MIC represents the lowest concentration of an antibiotic that inhibits visible bacterial growth (Figure 86). This value is crucial for determining appropriate antibiotic dosing in clinical settings.

The Minimum Bactericidal Concentration (MBC) is the lowest antibiotic concentration that kills 99.9% of the bacterial population. The MBC test extends the MIC assessment by subculturing from tubes or wells showing no visible growth onto antibiotic-free agar (Figure 86). This process determines whether bacteria have been just inhibited (bacteriostatic effect) or killed (bactericidal effect).

The relationship between MIC and MBC values provides insight into antibiotic action. For bactericidal antibiotics, MIC and MBC values are typically very similar. MBC values for bacteriostatic antibiotics are higher than MIC values.

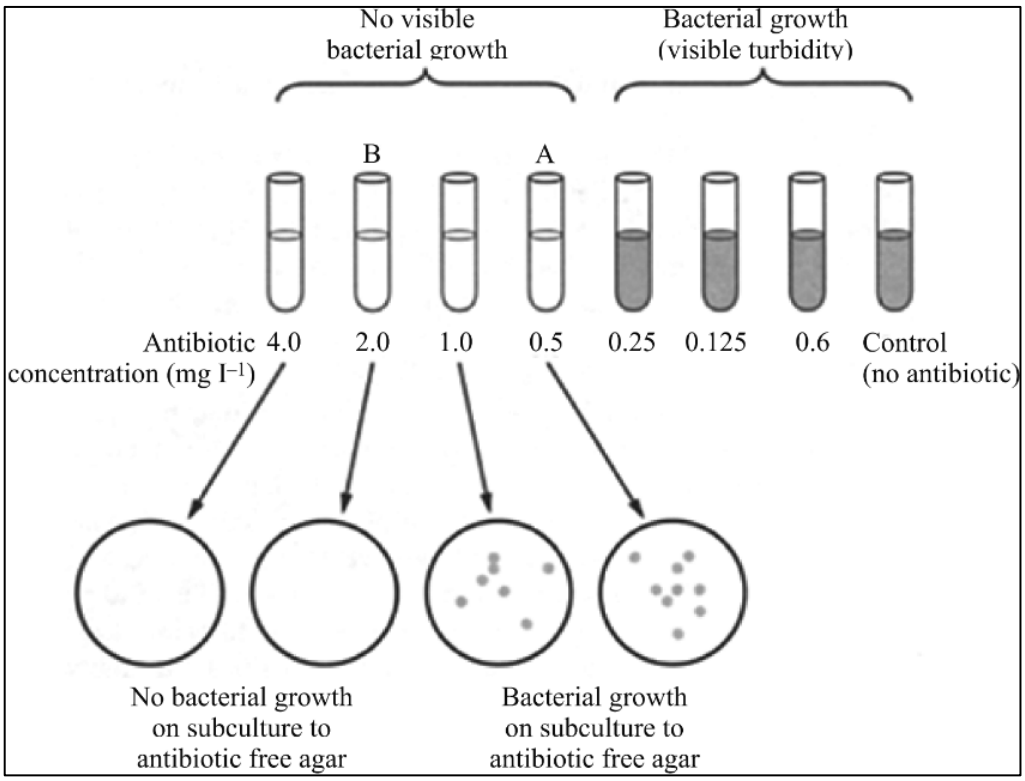


Figure 86. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determinations.

Chapter VI

Bacterial Taxonomy

Chapter VI. Bacterial Taxonomy

Bacterial taxonomy is a key field in microbiology that establishes the foundation for the organization, nomenclature, and classification of the vast diversity of bacteria. This chapter investigates the principles, methodologies, and applications of bacterial taxonomy, which is essential for comprehending the relationships between various bacterial groups and for precisely identifying bacterial species in many domains, including medicine, industry, and environmental science.

1. Introduction to Bacterial Taxonomy

Taxonomy (from the Greek words "*taxis*" = arrangement or order; "*nomos*" = law, to govern) is defined as the science of biological classification that arranges organisms into groups based on similarities and differences. In microbiology, taxonomy comprises three interrelated disciplines:

a) **Classification:** Arranging organisms into taxonomic groups (taxa) based on biochemical, physiological, genetic, and morphological properties.
b) **Nomenclature:** Assigning names to taxonomic units according to the International Code of Nomenclature of Bacteria (ICNB).
c) **Identification:** The practical process of determining that a particular isolate belongs to a recognized taxon.

2. Importance of Bacterial Taxonomy

Bacterial taxonomy serves several crucial purposes:

- ✓ Organizing vast knowledge about microorganisms in an accessible format.
- ✓ Enabling scientists to make predictions and formulate hypotheses based on knowledge of similar organisms.
- ✓ Placing microorganisms in meaningful groups with precise names for efficient scientific communication.
- ✓ Facilitating accurate identification of microorganisms, especially critical in clinical microbiology where treatment depends on correct pathogen identification.

3. Taxonomic Hierarchy

Taxonomic ranks form a hierarchical system where each lower level is contained within the one above it. A group of organisms at a specific rank is called a taxon (plural: taxa).

The main taxonomic ranks in prokaryotic taxonomy, in ascending order, are:

- **Species:** The most basic taxonomic group and the most specific category. It consists of a collection of related strains sharing many characteristics and showing high overall similarity.
- **Genus:** A collection of related species that share significant characteristics but remain distinct enough for separate classification. The plural of genus is genera.

- **Family**: A collection of similar genera, characterized by names that end with the suffix "-aceae" (e.g., Enterobacteriaceae).
- **Order:** A collection of similar families, with names ending in "-ales" (e.g., Enterobacteriales).
- **Class:** A collection of similar orders, typically with names ending in "-ia" (e.g., Gammaproteobacteria).
- **Phylum:** A collection of similar classes representing major evolutionary lineages. The plural of phylum is phyla.
- **Kingdom:** A collection of similar phyla or divisions.
- **Domain:** The highest taxonomic rank in the current classification system, representing a collection of similar kingdoms.

The following Table represents the taxonomic classification of *E. coli*.

Table 4. Taxonomic classification of the bacteria *Escherichia coli*.

Domain	Bacteria
Kingdom	Eubacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Enterobacteriales
Family	Enterobacteriaceae
Genus	*Escherichia*
Species	*Escherichia coli*

4. The Three Domains of Life

Based on comparative studies of ribosomal RNA sequences, the American microbiologist Carl Woese proposed a classification system dividing all living organisms into three domains: Bacteria, Archaea, and Eukarya (Figure 87).

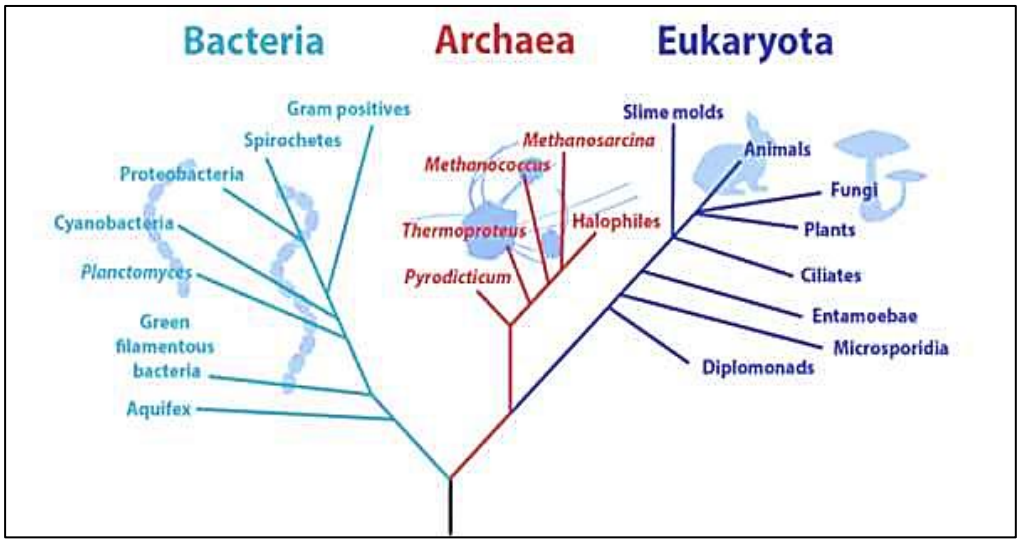


Figure 87. The three-domain system.

4.1. Domain Bacteria

Characterized by unique rRNA sequences distinct from Archaea and Eukarya, cell membranes with unbranched fatty acid chains attached to glycerol by ester linkages, cell walls containing peptidoglycan, and sensitivity to many antibiotics.

4.2. Domain Archaea

Characterized unique rRNA sequences distinct from Bacteria and Eukarya, cell membranes with branched hydrocarbon chains attached to glycerol by ether linkages, cell walls lacking peptidoglycan, insensitivity to some antibiotics that affect Bacteria, and the ability to thrive in extreme environments.

Archaea are divided into three main groups:

- **Methanogens:** Strict anaerobes that produce methane.
- **Extreme Halophiles:** Require high salt concentrations.
- **Thermoacidophiles:** Live in hot and acidic environments.

4.3. Domain Eukarya

Eukarya has eukaryotic cells with a membrane-bound nucleus. Organisms of this domain have membranes that contain unbranched fatty acid chains and glycerol. They do not have peptidoglycans on their cell wall. This domain includes all eukaryotic organisms: animals, plants, fungi, and protists.

5. Criteria used in bacterial taxonomy

For classifying and identifying bacteria, various characteristics are studied. These characteristics can be classified into two main groups:

5.1. Classical (Phenotypic) characteristics

The phenetic approach, which dominated bacterial taxonomy through much of the 20th century, classified bacteria based on observable traits (phenotypes) including:

5.1.1. Morphological characteristics

Morphological characteristics include the study of cell shape (cocci, bacilli, spirilla, etc.), cell size and arrangement (pairs, chains, clusters), colonial morphology, ultrastructural features (flagella, pili, fimbriae, capsule), spore formation, shape, and location, motility mechanisms, staining behavior (Gram staining, acid-fast staining), and cell inclusions.

5.1.2. Physiological and biochemical characteristics

They include the study of various tests, such as carbon and nitrogen sources, energy sources and metabolism, cell wall constituents, fermentation products, enzyme activities (catalase, oxidase), growth parameters (temperature, pH, osmotic pressure), oxygen requirements, nutritional type, and antibiotic sensitivity.

5.1.3. Chemical characteristics

Including the study of cell wall composition, cell membrane components, cytoplasmic structural components, and photosynthetic pigments.

5.1.4. Antigenic Characteristics (Serotyping)

Serotyping is based on reactions between specific antibodies and bacterial antigens. It is particularly important in epidemiological studies and can be used to differentiate strains within a species.

5.1.5. Ecological characteristics

Ecological characteristics are based on habitat and distribution, environmental relationships (symbiosis, parasitism), and pathogenicity in specific hosts.

5.2. Molecular (Genotypic) characteristics

Molecular characteristics provide more precise information about relationships between bacteria. These include:

5.2.1. Nucleic acid base composition (GC%)

In this technique, the DNA base composition is determined by measuring the Guanine and Cytosine content (GC%) or Chargaff coefficient, which is calculated using the following formula:

$$\mathbf{GC\%} = \frac{\mathbf{G + C}}{\mathbf{G + C + A + T}} \times \mathbf{100}$$

The GC% varies widely among prokaryotes (25-80%) compared to eukaryotes (30-50%). Within a species, the GC% remains relatively constant, with variations typically less than 2.5%. Organisms that differ in their GC% by more than 10% are generally not closely related. However, GC% alone can be misleading as it only provides information about the overall composition and not the sequence of DNA bases.

5.2.2. Nucleic acid hybridization

Nucleic acid hybridization is used to compare genome similarities between organisms. In this technique, double-stranded DNAs (dsDNAs) are heated to form single-stranded DNAs (ssDNAs). The ssDNAs are allowed to cool at a temperature 25°C below the melting temperature (Tm). At this temperature, complementary base sequences reassociate to form double-stranded DNA (Figure 88).

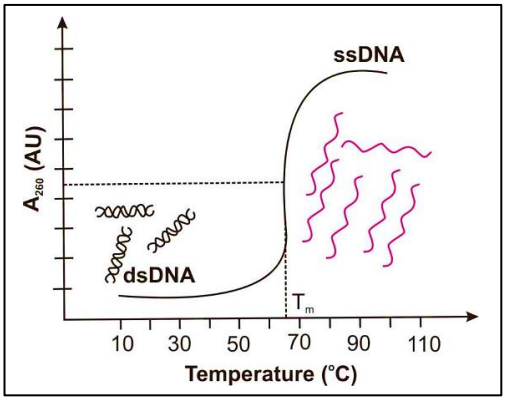


Figure 88. Determination of melting temperature (Tm).

The reassociation of complementary sequences is measured and the degree of hybridization indicates genetic similarity (Figure 89):

- ✓ Organisms showing at least 70% (≥70%) similarity based on hybridization are considered members of the same species.
- ✓ A degree of at least 25% (≥25%) suggests that two bacteria should reside in the same genus.
- ✓ A degree of 10% (≤10%) or less indicates distant taxonomic relationship.

The following figure illustrates the different steps of nucleic acid hybridization.

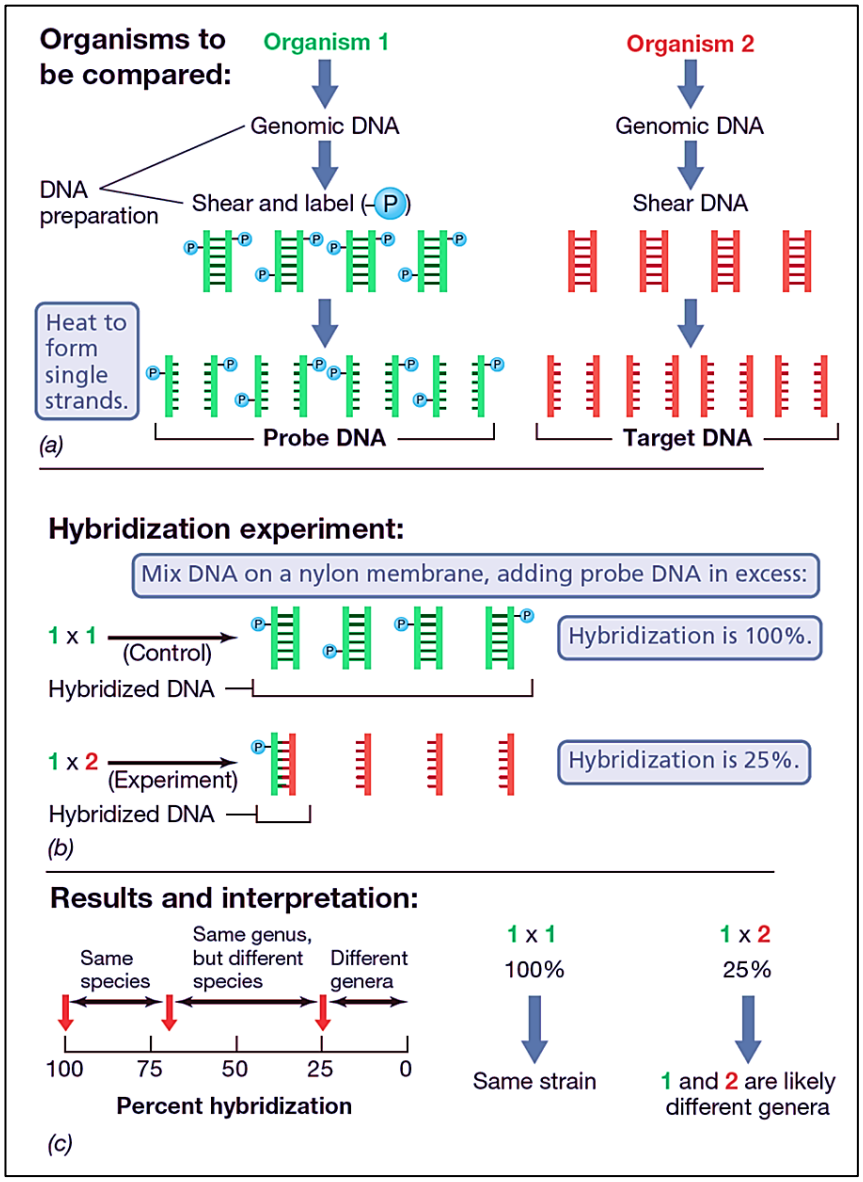


Figure 89. Genomic hybridization as a taxonomic tool. **(a):** Genomic DNA is isolated from the organisms to be compared and then sheared and denatured. Probe DNA is prepared from organism 1 by shearing, denaturing, labeling the DNA (shown here as radioactive phosphate). **(b):** sheared single-stranded target DNA from each genome is mobilized on a membrane and then hybridized with the labeled probe DNA from organism 1. Radioactivity in the hybridized DNA is measured. (c): Radioactivity in the control (organism 1 DNA hybridizing to itself) is taken as the 100% hybridization value.

5.2.3. Nucleic acid sequencing

Nucleic acid sequencing allows direct comparison of genomic structures. The 16S rRNA gene (or 16S rDNA) has been extensively used for sequence-based evolutionary analysis because:

- ✓ It is universal.
- ✓ It has a highly conserved nature.
- ✓ It has a moderate and reasonable length.
- ✓ It contains both conserved regions (for comparing distantly related organisms) and variable regions (for comparing closely related organisms).

The 16S rRNA gene consists of conserved sequences flanking several variable regions (V1 to V9) (Figure 90). The conserved regions are used to design universal primers for PCR amplification, while the variable regions enable comparison between closely related microbes. By convention, organisms showing more than 97% similarity in rRNA gene sequence can generally be considered members of the same species, although this criterion alone is not always sufficient.

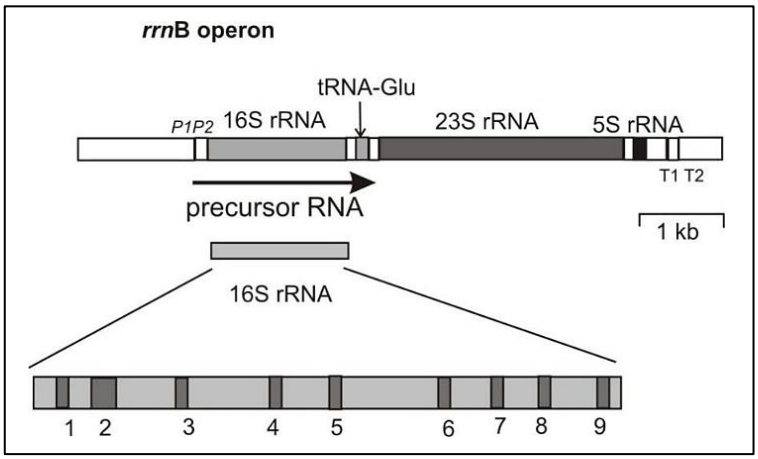


Figure 90. The 16S rRNA gene and its conserved sequences flanking several variable regions (V1 to V9).

6. Nomenclature

Nomenclature is the assignment of names to taxonomic units or groups based on specific rules and guidelines. Bacterial nomenclature follows the binomial system introduced by Carl Linnaeus in 1753, where every organism is assigned two names: the first name indicates the genus (generic name), and the second name indicates the species (specific epithet). For example: *Escherichia coli*.

- **Rules of bacterial nomenclature**

Bacterial nomenclature follows the International Code of Nomenclature of Bacteria (ICNB), which establishes several important rules:

- ✓ The names are written in italics or are underlined.
- ✓ The genus name is always capitalized, while the species name is always lowercase.
- ✓ Species names may be followed by subspecies names (e.g., *Salmonella enterica* subsp. *enterica* serovar *Typhimurium*).

- ✓ Family names end with "-aceae" and order names with "-ales".
- ✓ Names may be derived from various sources, including morphological characteristics (e.g., *Bacillus* for rod-shaped, *Streptococcus* for spheres in chains), distribution (e.g., *Aerococcus* for air, *Enterococcus* for gut), pathogenicity (e.g., *S. pneumoniae* for pneumonia), biochemical characteristics (e.g., *Chromobacterium* for producing pigment), or names of scientists or geographical locations.

The International Committee on Systematics of Prokaryotes (ICSP) publishes rules for naming prokaryotes in the Bacteriological Code. New bacterial names must be reviewed and published in the International Journal of Systematic and Evolutionary Microbiology (IJSEM) before official recognition.

7. Bergey's Manual of Systematic Bacteriology

Bergey's Manual of Systematic Bacteriology is the most comprehensive reference for bacterial taxonomy (Figure 91). It was first published in 1984 as a successor to Bergey's Manual of Determinative Bacteriology (first published in 1923).

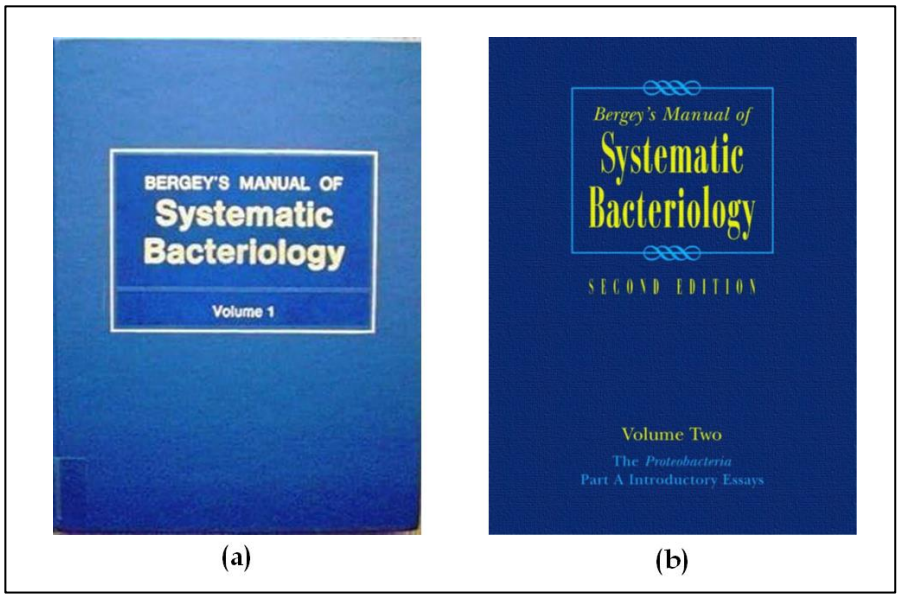


Figure 91. Bergey's Manual of Systematic Bacteriology. **(a):** The first edition (volume One); **(b):** The second edition (Volume Two).

- **First Edition (1984-1989):**

The first edition consists of four volumes covering:

- **Volume One (1984):** Gram-negative bacteria of general, medical, or industrial importance.
- **Volume Two (1986):** Gram-positive bacteria other than Actinomycetes.
- **Volume Three (1989):** Archaea, Cyanobacteria, and remaining Gram-negative bacteria.
- **Volume Four (1989):** Actinomycetes.

This edition classified the Kingdom Prokaryotae into four divisions:

a) **Gracilicutes:** Gram-negative cell wall bacteria.
b) **Firmicutes:** Gram-positive cell wall bacteria.
c) **Tenericutes:** Bacteria without cell walls.
d) **Mendosicutes:** Bacteria lacking peptidoglycan (similar to Archaea).

- **Second Edition (2001-2012):**

The second edition consists of five volumes based on phylogenetic relationships:

- **Volume One (2001):** Archaea and deeply branching and phototrophic bacteria.
- **Volume Two (2005):** Proteobacteria.
- **Volume Three (2009):** Firmicutes.
- **Volume Four (2010):** Bacteroidetes, Spirochaetes, and other phyla.
- **Volume Five (2012):** Actinobacteria.

While the first edition was phenetic, the second edition is based on phylogenetic characters, such as DNA, RNA, and protein sequences.

8. Methods of Bacterial Identification

8.1. Classical methods

Classical methods of bacterial identification rely on phenotypic characteristics:

- **Microscopic Observation:** Direct examination of bacterial morphology, arrangement, motility, and spore formation.
- **Staining Techniques**: Various staining procedures help identify structural features, including Gram staining, acid-fast staining, spore staining, and capsule staining.
- **Cultural characteristics:** Observation of growth patterns on different media, such as colony morphology (size, shape, color, texture), hemolysis patterns on blood agar, and growth on selective and differential media.
- **Biochemical tests:** These tests detect specific metabolic activities, such as enzyme production (catalase, oxidase, etc.), indole production, methyl red test, Voges-Proskauer, citrate utilization, urease test, sugar fermentation, and nitrate reduction.
- **Automated identification systems:** Miniaturized biochemical test systems like API (Analytical Profile Index) strips, which contain multiple tests in a single device (Figure 92). After inoculation and incubation, the results are converted into a numerical profile that can be matched to a database for identification.

Figure 92. API 20 E identification system reactions.

8.2. Molecular methods

8.2.1. PCR and DNA Sequencing

Polymerase chain reaction (PCR) amplifies specific DNA sequences, which can then be sequenced. The 16S rRNA gene is commonly used for bacterial identification due to its universal presence and combination of conserved and variable regions.

8.2.2. DNA-DNA Hybridization

This technique measures the degree of genetic similarity between different bacterial strains. It involves denaturing DNA from two different sources, allowing the single strands to reassociate, and measuring the extent of hybridization.

8.2.3. MALDI-TOF mass spectrometry

This approach analyzes the protein composition of bacterial cells, producing a characteristic spectrum that can be compared to a database for identification.

9. Definition of Bacterial Species

The current definition of a bacterial species is based on DNA-DNA hybridization studies. According to the International Committee on Systematics of Prokaryotes, a bacterial species is defined as a group of strains that show 70% or greater DNA-DNA relatedness and have a thermal stability of hybrid (ΔTm) of 5°C or less. This definition, while practical, has limitations and continues to evolve as new molecular techniques become available.

10. Major Bacterial Groups

According to the second edition of Bergey's Manual of Systematic Bacteriology, bacteria are divided into two domains (Bacteria and Archaea) and multiple phyla, including:

10.1. Proteobacteria

It is the largest and most diverse phylum, divided into five classes:

- Alphaproteobacteria (e.g., *Rhizobium*, *Rickettsia*).
- Betaproteobacteria (e.g., *Neisseria*, *Bordetella*).
- Gammaproteobacteria (e.g., Enterobacteriaceae, Pseudomonadaceae, Vibrionaceae).
- Deltaproteobacteria (e.g., *Myxococcus*, sulfate-reducing bacteria).
- Epsilonproteobacteria (e.g., *Helicobacter*, *Campylobacter*).

10.2. Firmicutes

Gram-positive bacteria with low G+C content, including:

- Bacilli (*Bacillus, Staphylococcus, Streptococcus, Lactobacillus*).
- Clostridia (*Clostridium*).

10.3. Actinobacteria

Gram-positive bacteria with high G+C content, including:

- *Mycobacterium*
- *Streptomyces*
- *Corynebacterium*

10.4. Bacteroidetes

Includes many anaerobic Gram-negative bacteria found in the human gut.

10.5. Cyanobacteria

Photosynthetic bacteria that produce oxygen.

10.5. Other Significant Phyla

10.5.1. Spirochaetes

Spiral-shaped bacteria with unique flagella.

10.5.2. Tenericutes

Bacteria lacking cell walls, including Mycoplasma.

10.5.3. Chlamydiae

Obligate intracellular pathogens.

Bacterial taxonomy continues to evolve as a discipline, balancing the need for stability in nomenclature with the incorporation of new scientific discoveries. The integration of phenotypic characteristics with increasingly sophisticated molecular and genomic analyses has created a robust framework for understanding bacterial diversity. As technology advances, bacterial taxonomy will continue to refine our understanding of microbial relationships while maintaining its practical utility for identification.

Chapter VII

Role of Microorganisms in Industry, Environment and Health

Chapter VII. Role of Microorganisms in Industry, Environment and Health

1. The Role of Microorganisms in Industry

Microorganisms play a pivotal role in various industrial processes, contributing to the production of economically valuable products and services. This section outlines their applications, the types of microorganisms used, and the processes involved.

Microorganisms are employed in industries to produce a wide range of products, including:

- ✓ Microbial metabolites: These include primary metabolites like amino acids (e.g., L-glutamic acid, L-lysine) and secondary metabolites such as antibiotics (e.g., streptomycin, penicillin).
- ✓ Enzymes: Microbial enzymes like amylase, protease, and pectinase are widely used in food processing, textile industries, and pharmaceuticals.
- ✓ Food products: Fermentation processes involving microorganisms produce foods such as cheese, yogurt, bread, and vinegar.
- ✓ Beverages: Alcoholic beverages like wine and beer are produced using yeast fermentation.
- ✓ Organic acids: Examples include lactic acid, citric acid, and acetic acid.
- ✓ Biofuels: Microorganisms such as algae are used for biofuel production.
- ✓ Recombinant products: Genetic engineering enables the production of insulin and vaccines using microbial systems.

1.1. Microbial processes in industry

The term fermentation broadly refers to any process mediated by microorganisms to produce valuable products. Industrial microbiology encompasses:

- **Isolation and screening:** Microorganisms are isolated from natural habitats (e.g., soil or water) and screened for their ability to produce specific metabolites or enzymes.
- **Strain improvement:** Genetic manipulation techniques such as mutant selection or recombinant DNA technology are employed to enhance product yields.
- **Fermentation processes:** These include batch fermentation, continuous fermentation, fed-batch fermentation, solid-state fermentation (SSF), and submerged liquid fermentation (SLF). Each method is tailored to optimize microbial growth and product formation.

1.2. Properties of industrial microorganisms

Microorganisms used in industry must possess specific properties:

- ✓ High yield of desired products.
- ✓ Rapid growth and reproduction under large-scale conditions.

- ✓ Ability to grow in inexpensive media derived from industrial waste (e.g., corn steep liquor or whey).
- ✓ Non-pathogenic nature for safety reasons.
- ✓ Genetic stability and amenability to genetic manipulation for strain improvement.

1.3. Bioreactor

A bioreactor is a device or system designed to provide a controlled environment to support the growth and metabolic activity of microorganisms, cells, or enzymes for industrial production (Figure 94). These systems are pivotal in industrial microbiology for the production of a wide range of products, including biomass, enzymes, antibiotics, biofuels (e.g., bioethanol, biodiesel), and vaccines.

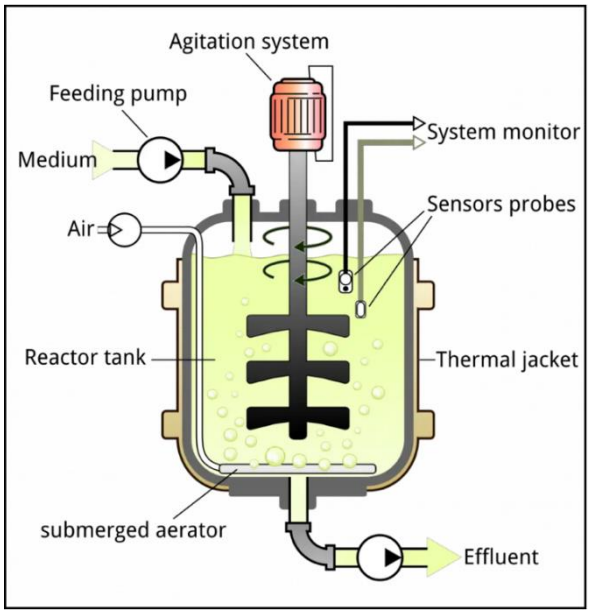


Figure 94. General structure of a bioreactor.

1.3.1. Key components of bioreactors

Each bioreactor is equipped with essential components to ensure optimal operation:

- ✓ **Agitation system:** Enhances mixing and oxygen transfer in liquid cultures.
- ✓ **Aeration system:** Supplies oxygen for aerobic processes using spargers or diffusers.
- ✓ **Temperature control:** Maintains optimal conditions using heating or cooling systems (jackets).
- ✓ **pH sensors:** Monitor and adjust pH levels to maintain microbial activity.
- ✓ **Sampling ports:** Allow periodic sampling for monitoring product yield and microbial growth.

1.3.2. Principles of bioreactor operation

The primary function of a bioreactor is to maintain optimal conditions for microbial growth and metabolic activity. This involves:

- ✓ **Temperature control:** Precise regulation to prevent thermal stress.
- ✓ **pH Maintenance:** Ensuring an environment conducive to enzymatic activity.
- ✓ **Aeration and agitation:** Supplying oxygen and mixing nutrients uniformly.
- ✓ **Sterility:** Preventing contamination by unwanted microorganisms.

By controlling these parameters, bioreactors facilitate efficient biotransformation and bioconversion processes.

1.4. Types of fermentation processes

Industrial fermentation methods are categorized based on operational strategies and microbial growth conditions (Table 5).

Table 5. Industrial fermentation methods

Type	Description	Applications	Advantages	Disadvantages
Batch Fermentation	Closed system with no nutrient addition after inoculation.	Alcohol, penicillin, yogurt.	Simple operation; low contamination risk.	Limited by substrate depletion; low productivity.
Continuous Fermentation	Fresh medium continuously added; spent medium removed (chemostat).	Ethanol, organic acids.	Steady-state conditions; higher productivity.	Complex control systems; contamination risk.
Fed-Batch Fermentation	Substrate added incrementally during fermentation.	Amino acids, recombinant proteins.	Controls substrate inhibition; extends productivity.	Requires precise nutrient monitoring.
Solid-State Fermentation (SSF)	Uses low-moisture substrates (e.g., wheat bran, rice).	Enzymes (amylase), citric acid, mushrooms.	High product concentration; low wastewater.	Limited to fungi/actinomycetes; heat management issues.
Submerged Fermentation (SLF)	Cells grown in liquid medium with agitation/aeration.	Antibiotics, vaccines, extracellular enzymes.	Uniform nutrient distribution ; scalable.	High energy costs for aeration/agitation.
Anaerobic Fermentation	Conducted without oxygen (e.g., Clostridium spp.).	Acetone, butanol, biogas.	No aeration costs; suitable for obligate anaerobes.	Slow growth rates; gas management required.
Aerobic Fermentation	Requires oxygen supply (e.g., stirred-tank bioreactors).	Vitamins, antibiotics, single-cell protein.	Supports high-density microbial growth.	High energy for aeration; foaming issues.

1.5. Downstream processing

The various processes used for the actual recovery of useful products from a fermentation or any other industrial process is called downstream processing. After fermentation, downstream processing involves:

- ✓ Separation of microbial biomass from the medium via filtration (e.g., filamentous fungi) or centrifugation (e.g., bacterial or yeast cells).
- ✓ Extraction and purification of the desired product (e.g., chromatography, ultrafiltration, or crystallization).
- ✓ Drying and stabilization for commercial use (e.g., lyophilization or immobilization).

1.6. Industrial applications of microorganisms

1.6.1. Microbial biomass production

Microbial biomass, often referred to as termed single-cell protein (SCP), involves cultivating microorganisms to generate a protein-rich biomass for human food, animal feed, or industrial substrates. This approach is particularly valuable for its ability to utilize inexpensive substrates, including agricultural and industrial byproducts, to produce high-quality protein efficiently.

a) Key Microorganisms

A variety of microorganisms are used in biomass production:

- **Yeasts**: *Candida utilis* (grown on molasses), *Saccharomyces cerevisiae* (brewer's yeast).
- **Bacteria**: including species like *Methylophilus methylotrophus* (grow on methanol-based substrates) and are used for animal feed application.
- **Filamentous fungi**: such as *Fusarium venenatum*, and *Aspergillus oryzae*.
- **Algae**: especially *Spirulina* spp., which are cultivated for their high protein content and rich nutritional profile.

b) Production process

- **Substrate preparation**: Inexpensive carbon sources (e.g., corn steep liquor, whey) are sterilized and supplemented with nitrogen (e.g., ammonium salts) and minerals to support microbial growth.
- **Fermentation**: Microorganisms are cultivated in aerobic bioreactors to maximize biomass yield. Aeration and agitation ensure efficient oxygen transfer and nutrient distribution.
- **Harvesting**: Microbial cells are separated from the fermentation medium via centrifugation or filtration techniques.
- **Post-processing**: Harvested biomass undergoes drying to improve shelf, and texturization (for food applications). It is sometimes processed further to reduce nucleic acid content for human consumption (to improve digestibility).

c) Applications of microbial biomass

- **Human food**: Mycoprotein from *F. venenatum* is used in meat substitutes due to its high protein content and fiber-like texture. Yeast extracts serve as flavor enhancers in soups and sauces.
- **Animal feed**: SCPs are used as a protein supplement for livestock, poultry, and aquaculture species due to their high digestibility and amino acid profile.
- **Industrial use**: Employed as starter cultures in fermentation processes or as biocatalysts.

1.6.2. Industrial production of primary metabolites

Primary metabolites are synthesized during the active growth phase (trophophase) and are directly involved in cellular metabolism. They include amino acids, vitamins, organic acids, and alcohols, which are critical for microbial growth and have widespread industrial applications.

a) Amino acid production

Amino acids such as L-glutamic acid and L-lysine are produced via microbial fermentation. Microorganisms, like *Corynebacterium glutamicum* and *Brevibacterium flavum* are key producers.

The production process typically involves fed-batch submerged fermentation, a method chosen for its ability to maintain optimal nutrient levels while minimizing inhibitory byproduct accumulation. In this system, glucose or molasses serves as the primary carbon source.

Strain optimization is critical for bypassing natural regulatory mechanisms like feedback inhibition. Auxotrophic mutants are one of the strategies used to bypass feedback inhibition. For example, phenylalanine auxotrophs of *C. glutamicum* accumulate precursors of the lysine biosynthesis pathway, as phenylalanine deprivation disrupts feedback regulation.

- **Applications**
  - ✓ **Food industry:** Flavor enhancers (monosodium glutamate, MSG) and nutritional supplements.
  - ✓ **Animal feed:** Lysine supplementation improves protein quality.

b) Vitamin production

Vitamins are essential micronutrients critical for human and animal health, and their microbial biosynthesis offers a sustainable and cost-effective alternative to chemical synthesis. Two industrially significant vitamins produced via microbial fermentation are vitamin B12 (cobalamin) and riboflavin (vitamin B2).

- **Vitamin B12 production**

Vitamin B12 is primarily synthesized by specific bacteria, notably *Pseudomonas denitrificans* and *Propionibacterium freudenreichii*. These bacteria are metabolic specialists optimized for high-yield cobalamin production. *P. freudenreichii* is particularly favored in food applications due to its GRAS (Generally Recognized As Safe) status.

B12 biosynthesis occurs under aerobic conditions in stirred-tank bioreactors to ensure optimal oxygen transfer, which is critical for the oxygen-dependent steps in cobalamin synthesis. Cost-effective substrates like corn steep liquor (a nitrogen-rich byproduct of corn processing) and molasses are used to reduce production costs. The media is often supplemented with cobalt, a central atom in the B12 molecule.

The purification process involves filtration, solvent extraction, and crystallization. However, the complex structure of B12 necessitates careful handling to retain stability during purification.

Industrial vitamin B12 has various applications, including:

- ✓ Pharmaceuticals: B12 supplements are critical for treating deficiencies linked to anemia and neurological disorders.
- ✓ Food Fortification: Added to plant-based foods (e.g., cereals, plant-based milk) and dairy products to enhance nutritional profiles, especially in vegan diets.

- **Riboflavin (vitamin B2) production**

Riboflavin is industrially produced using the filamentous fungus *Ashbya gossypii* and the bacteria *B. subtilis*. The fungus *A. gossypii* is preferred for its high riboflavin yields, while *B.subtilis* is valued for rapid growth and genetic tractability.

The fermentation process is conducted in aerobic stirred-tank bioreactors to maximize oxygen availability, which enhances fungal growth and riboflavin secretion. Molasses and corn steep liquor serve as primary substrates, providing carbohydrates, nitrogen, and trace elements. *B. subtilis* strains are often genetically modified to overexpress riboflavin biosynthesis genes.

Applications of riboflavin include:

- ✓ Pharmaceuticals: Used in multivitamin supplements and injectable formulations.
- ✓ Food Industry: Fortifies cereals, infant formulas, and dairy products.
- ✓ Animal Feed: Added to livestock feed to improve growth and health.

c) Organic acid production

Organic acids are vital primary metabolites produced by microorganisms during their active growth phase (trophophase). These compounds have widespread industrial applications, including food preservation, pharmaceuticals, biodegradable plastics, and chemical feedstocks.

Key organic acid and their microbial producers include:

- **Citric acid:** One of the most widely produced organic acids globally, primarily used in the food and beverage industry. It is synthesized by filamentous fungi such as *Aspergillus niger* and yeasts like *Candida* spp.
- **Lactic acid:** Produced by bacteria such as *Lactobacillus* spp. (e.g., *L. delbrueckii, L. casei*) and fungi like *Rhizopus oryzae*. The process involves anaerobic or microaerophilic fermentation using glucose or lactose derived from whey as the substrate.

- **Acetic acid:** Primarily produced by acetic acid bacteria such as Acetobacter aceti and Gluconacetobacter xylinus.
- **Butyric acid:** Produced anaerobically by clostridia such as *C. butyricum* and *C. acetobutylicum*. These microorganisms utilize starch or lignocellulosic waste (e.g., corn husks) as substrates under strictly anaerobic conditions.
- **Fumaric acid:** Synthesized by filamentous fungi such as Rhizopus arrhizus and R. oryzae. Solid-state fermentation (SSF) using agricultural residues like wheat bran or rice husks is commonly employed for its production.

Below is a structured table summarizing the industrial production of key organic acids, including their microbial producers, production processes, applications, and challenges.

Table 6. Industrial production of organic acids via microbial fermentation

Organic acid	Microorganisms	Production process	Applications
Citric acid	*Aspergillus niger, Candida* spp.	Submerged liquid fermentation (SLF) is the primary method.	Food acidulants (soft drinks, candies, and preserved foods), pharmaceuticals (effervescent tablets and anticoagulants), and biodegradable polymers.
Lactic acid	*Lactobacillus* spp., *Rhizopus oryzae*	Batch or fed-batch fermentation methods are used.	- Food preservation (dairy products and pickled vegetables), biodegradable plastics (PLA), and pharmaceuticals.
Acetic acid	*Acetobacter aceti*, *Gluconacetobacter xylinus*	Aerobic fermentation of ethanol is used in submerged bioreactors.	vinegar production and chemical feedstocks like vinyl acetate.
Butyric acid	*Clostridium butyricum, C. acetobutylicum*	Anaerobic fermentation using starch or lignocellulosic waste.	Food flavoring agent (cheese and butter formulations), Pharmaceuticals (precursor for anticancer butyrate derivatives).
Fumaric acid	*Rhizopus arrhizus, R. oryzae*	Solid-state fermentation (SSF) using agricultural residues like wheat bran or rice husks.	Food acidulants (beverages and baking powders), Polymer production (unsaturated polyester resins for manufacturing durable materials).

1.6.3. Industrial production of secondary metabolites: Antibiotics

Antibiotics are among the most critical secondary metabolites produced by microorganisms, revolutionizing medicine by treating bacterial infections and saving millions of lives. These compounds are synthesized during the stationary phase (idiophase) of microbial growth, often triggered by nutrient depletion or environmental stress. Unlike primary metabolites, antibiotics are

not essential for microbial survival but confer ecological advantages, such as inhibiting competitors or modulating symbiotic relationships.

Industrially, antibiotics like penicillin (which targets Gram-positive bacteria by disrupting cell wall synthesis), streptomycin (effective against tuberculosis and Gram-negative pathogens), tetracyclines (broad-spectrum antibiotics used for respiratory and skin infections), and cephalosporins (efficient against penicillin-resistant strains) are produced through controlled fermentation processes using filamentous fungi and actinomycetes (Table 7).

Table 7. Key antibiotics and producers

Antibiotic	Microbial source	Applications
Penicillin	*Penicillium chrysogenum*	Treats bacterial infections (e.g., streptococcal infections).
Streptomycin	*Streptomyces griseus*	Used against tuberculosis and Gram-negative pathogens.
Tetracyclines	*Streptomyces aureofaciens*	Broad-spectrum antibiotic for respiratory and skin infections.
Cephalosporins	*Acremonium chrysogenum*	Effective against penicillin-resistant bacteria.

The production process of antibiotic biosynthesis involves fed-batch fermentation to prolong the stationary phase, where secondary metabolite production peaks. Media optimization is critical: low nitrogen and phosphate levels often induce antibiotic synthesis. For example, penicillin production requires phenylacetic acid as a precursor. Strain improvement through mutagenesis (UV or chemical agents) and recombinant DNA technology enhances yields. *P. chrysogenum* mutants, developed over decades, now produce penicillin at scales exceeding 100,000 liters, reducing costs through economies of scale.

1.6.4. Industrial enzyme production

Microbial enzymes are indispensable in various industries due to their specificity, efficiency, and environmental sustainability (Table 8). These enzymes catalyze biochemical reactions under mild conditions, reducing energy consumption and chemical usage. They are derived from microorganisms such as bacteria, fungi, and yeasts, and play a vital role in processes ranging from food production to pharmaceuticals.

After fermentation, enzymes are extracted from the culture medium or microbial cells through filtration or centrifugation. Purification techniques such as ultrafiltration, chromatography, or crystallization are employed based on the required enzyme purity level. Stabilization methods like lyophilization or immobilization ensure prolonged enzyme activity during storage.

Microbial enzymes can be immobilized onto solid supports (e.g., alginate beads) for reuse in continuous processes such as glucose isomerization during high-fructose corn syrup production. Immobilization also enhances enzyme stability under industrial conditions.

Table 8. Major enzyme classes and applications.

Enzyme	Microbial source	Production process	Applications
Amylases	*Aspergillus niger, Bacillus* spp.	Submerged liquid fermentation (SLF) in aerated stirred-tank bioreactors. Fed-batch systems to avoid substrate inhibition.	Food (Starch hydrolysis for glucose syrups and bioethanol production), and textile.
Proteases	*Bacillus subtilis, Streptomyces*	Submerged liquid fermentation for extracellular protease production.	Detergents, leather processing, cheese production
Lipases	*Candida rugosa, Rhizopus* spp.	Solid-state fermentation (SSF) using low-moisture substrates like wheat bran.	Food flavoring, biodiesel synthesis, and pharmaceuticals (Synthesis of chiral intermediates for drugs).
Cellulases	*Trichoderma reesei*	Submerged liquid fermentation with lignocellulosic waste as substrate.	Biofuel production, textile processing
Pectinases	*Aspergillus* spp.	Solid-state fermentation using agricultural residues like orange peels or wheat bran.	Fruit juice clarification, wine production
Glucose isomerase	*Streptomyces* spp.	Continuous immobilized enzyme systems for high-fructose corn syrup production.	Food (Conversion of glucose to fructose for sweeteners like high-fructose corn syrup).

2. The Role of Microorganisms in Health

2.1. Pathogenicity

2.1.1. Definition of pathogenicity

Pathogenicity refers to the ability of microorganisms to cause disease in a host organism. This property is determined by various microbial factors that enable the microbe to establish infection, multiply, and cause damage to host tissues. Pathogenicity is a qualitative property—a microorganism either can cause disease or it does not. A pathogen is, therefore, any microorganism (e.g., bacteria, virus, fungus) that can cause disease in a host.

Virulence, while related to pathogenicity, is a quantitative measure of the degree of pathogenicity exhibited by a microorganism. It reflects the severity of disease caused by the pathogen and can be measured by parameters such as the infectious dose (ID50) or lethal dose (LD50). Highly virulent pathogens cause severe disease even in small numbers, while less virulent ones may require larger populations to produce clinical symptoms.

2.1.2. Mechanisms of pathogenicity

A microorganism can cause disease through mechanisms such as adhesion, invasion, and immune evasion (Figure 95).

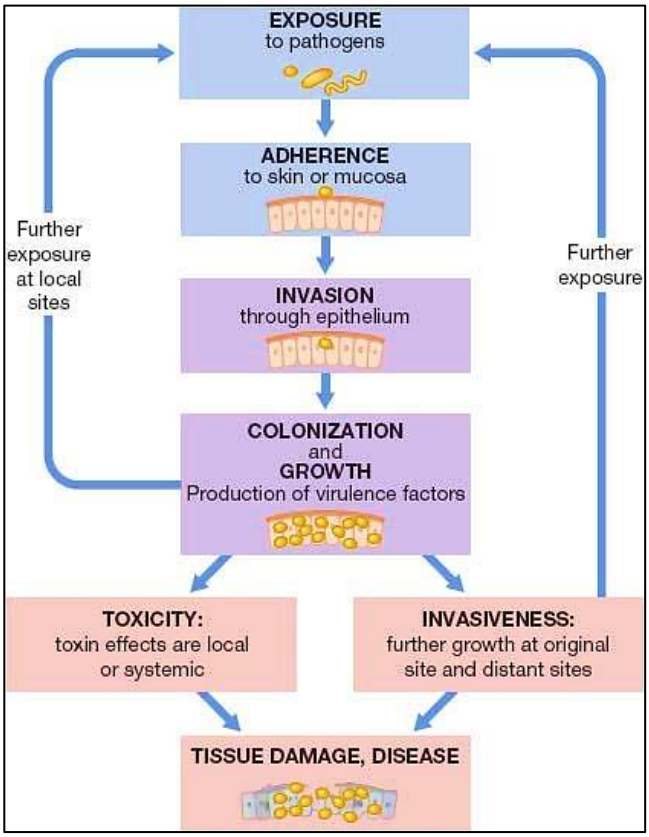


Figure 95. Mechanisms of pathogenicity.

a) Adherence to host cells

Microbial adherence to host tissues is the first critical step in infection pathogenesis. This involves specific interactions between microbial adhesins (including pili/fimbriae) and host cell receptors. For example, *E. coli* expresses P fimbriae that bind to galactose-containing glycolipids on uroepithelial cells, while *S. aureus* produces multiple adhesins (fibronectin-binding proteins, collagen-binding proteins), enabling attachment to various tissues. Biofilm formation represents an advanced adherence form where microorganisms create a protective matrix that enhances persistence by providing resistance to antimicrobials and host defenses.

b) Invasion and colonization

After adherence, pathogens penetrate tissues using extracellular enzymes that degrade cellular components. Hyaluronidase (produced by *S. pyogenes*) degrades hyaluronic acid in connective tissues, while collagenase breaks down collagen, and phospholipases disrupt cell membranes. Some pathogens have evolved sophisticated intracellular survival mechanisms - L. monocytogenes produces listeriolysin O to escape from phagosomes into the cytoplasm, while *M. tuberculosis* inhibits phagosome-lysosome fusion, creating a protected niche within macrophages.

c) Immune evasion

Pathogens employ various strategies to evade host immune responses. Polysaccharide capsules (like those of *S. pneumoniae*) inhibit phagocytosis by preventing opsonization, with capsule thickness directly correlating with virulence.

Antigenic variation is another sophisticated strategy. *N. gonorrhoeae* alters the antigenic composition of its pili and outer membrane proteins through genetic recombination, presenting a moving target to the adaptive immune system.

Some pathogens also produce proteases that degrade host immune molecules, such as *S. aureus* producing staphylokinase that cleaves IgG antibodies.

2.1.3. Toxin production

a) Endotoxins

Components of Gram-negative bacteria found in the outer membrane. The lipid portion of lipopolysaccharides (LPS), called lipid A, acts as an endotoxin. Most endotoxins are lipids. They are liberated during bacterial lysis after death. Major sites of action for endotoxins are macrophages. Bacterial lysis in macrophages causes a release of these endotoxins (LPS) from the surface of Gram-negative bacteria in the form of small pieces, which bind to LPS-binding protein in the plasma. They are extremely dangerous to the extent that they can cause severe damage to the organism. Examples include endotoxins from *E. coli, S. typhi, Shigella*, and *V. cholerae.*

b) Exotoxins

Proteins secreted by bacteria that act enzymatically or through direct interaction with host cells. They are heat-labile proteins, mainly secreted by some rare species of bacteria. Once the toxins are liberated, they diffuse into the surrounding medium and cause harm to the host cells either by disrupting the normal functioning of the cell or by directly destroying the cells. Examples include Neurotoxins produced by *C. botulinum* (botulism) and *C. tetani* (tetanus), enterotoxins produced by *V. cholerae* (cholera toxin).

The major differences between endotoxins and exotoxins are summarized in the Table below:

Table 9. Differences between endotoxins and exotoxins.

Characteristics	Endotoxins	Exotoxins
Definition	Structural components of Gram-negative bacteria (Lipopolysaccharides - LPS).	Proteins secreted by bacteria.
Source	Found in the outer membrane of Gram-negative bacteria.	Produced by both Gram-positive and Gram-negative bacteria.
Release	Released during bacterial cell lysis or growth.	Actively secreted by living bacteria.
Heat Stability	Heat-stable.	Heat-labile.

Table 9. Continued.

Characteristics	Endotoxins	Exotoxins
Toxicity	Moderately toxic; causes fever and inflammation.	Highly toxic; specific effects on target cells or organs.
Immunogenicity	Weakly immunogenic; does not elicit strong immune responses.	Strongly immunogenic; stimulates antibody production.
Detection	Detected using Limulus lysate assay.	Neutralized by antitoxins (antibodies).
Clinical Use	No toxoid vaccines are available.	Can be converted into toxoids for vaccines (e.g., diphtheria, tetanus).
Examples	LPS from *E. coli, Salmonella, Shigella*.	Tetanus toxin, botulinum toxin, cholera toxin.

2.2. Bacterial infection

Bacteria are omnipresent and crucial for maintaining our environment. Only a small percentage of global bacteria cause infection and disease. However, these infections have a large impact on public health.

2.2.2. Bacterial infection transmission

2.2.2.1. Overview of transmission routes

Transmission refers to the process by which pathogens disseminate from a source to a susceptible host. Understanding these dynamics is crucial for implementing effective control strategies against infectious diseases.

Transmission routes are classified as direct (immediate transfer from an infected to a susceptible host) or indirect (transfer via contaminated intermediaries, such as air, water, food, or vectors).

2.2.2.2. Transmission routes

a) Airborne transmission

It involves pathogen dissemination by respiratory droplets (>5 µm, travel short distances) or aerosols (<5 µm, remain suspended longer and travel farther). Examples include *M. tuberculosis* (tuberculosis), which is transmitted by infectious aerosols (can reach alveoli when inhaled) generated by infected individuals during coughing, and *S. pyogenes* (strep throat), which transmits via larger respiratory droplets during talking, coughing, or sneezing.

b) Waterborne transmission

It occurs when pathogens contaminate water sources used for drinking, food preparation, or recreation. *V. cholerae* (cholera) is a classic example of a waterborne pathogen. It spreads through fecally contaminated water sources, colonizing the small intestine and producing cholera toxins that cause severe diarrhea and dehydration.

c) Foodborne transmission

It involves consuming food contaminated with pathogens or their toxins. *Salmonella* causes salmonellosis through contaminated eggs, poultry, and meat products, invading intestinal epithelial cells and causing inflammation and diarrhea. *Listeria monocytogenes* (listeriosis) can grow at refrigeration temperatures in ready-to-eat foods (e.g., soft cheeses), causing severe disease in pregnant women, newborns and immunocompromised individuals.

d) Vector-borne transmission

It involves pathogen transfer between hosts by arthropod vectors like mosquitoes, ticks, and fleas. *Yersinia pestis* (plague) is transmitted by fleas. The bacteria blocks the fleas' digestive tracts, causing them to regurgitate bacteria into the wound during feeding, thus transmitting the pathogen to a new host. *Borrelia burgdorferi* (Lyme disease) is transmitted by Ixodes ticks where the spirochetes enter the salivary glands during feeding, from where it enters the new host.

e) Direct contact transmission

It occurs through physical contact with infected individuals, their bodily fluids, or contaminated surfaces. Methicillin-resistant *S. aureus* (MRSA) spreads in healthcare settings through contact with infected individuals, contaminated surfaces or medical equipment. The bacteria's ability to survive on dry surfaces for extended periods facilitates its transmission. Similarly, *Treponema pallidum* (syphilis) spreads through direct contact with infectious lesions during sexual activity.

f) Vertical transmission

It involves pathogen passage from mother to child during pregnancy, childbirth, or breastfeeding. *T. pallidum* can cross the placenta, causing congenital syphilis. *L. monocytogenes* can also cross the placental barrier, causing severe neonatal infections, including meningitis and sepsis. Group B *Streptococcus* can transmit to newborns during birth, potentially causing neonatal sepsis or meningitis.

2.2.2.3. Factors influencing transmission

Pathogen transmission is influenced by multiple factors, broadly categorized into environmental and host factors.

a) Environmental factors

They impact pathogen survival outside the host and subsequent transmission. Temperature affects microbial replication rates and survival. Humidity influences the survival of airborne pathogens. Sanitation infrastructure, including access to clean water and proper waste disposal, directly impacts the prevalence of enteric pathogens transmitted through the fecal-oral route.

b) Host factors

Population immunity levels determine the pool of susceptible individuals available for infection. Behavioral factors, such as hand hygiene practices, food handling, and sexual activities, influence exposure risk. Population density affects contact rates between individuals, with higher densities facilitating rapid spread of directly transmitted pathogens.

2.2.3. Clinical relevance and preventions

a) Impact on public health

Despite advancements in antimicrobial therapy, bacterial infections remain a global health challenge, particularly in low- and middle-income countries.

Antibiotic resistance, fueled by multidrug-resistant organisms such as MRSA, carbapenem-resistant Enterobacteriaceae, and extensively drug-resistant tuberculosis, limits treatment options and increases healthcare costs. Inappropriate antibiotic use and declining antibiotic development contribute to this issue.

b) Prevention strategies

Effective prevention of bacterial infections requires a multifaceted approach targeting various aspects of the transmission chain:

- **Vaccination programs:** Vaccines like pneumococcal conjugate (for *S. pneumoniae*) and *Haemophilus influenzae* type b have reduced the incidence of invasive diseases.
- **Improved sanitation and hygiene practices:** Hand hygiene, access to clean water, and food safety protocols can reduce the dissemination of pathogens.
- **Vector control measures:** Insecticide-treated nets for malaria prevention and environmental management can also help control tick populations.

2.2.4. Case studies

a) Cholera

Cholera, caused by *V. cholerae*, remains a significant public health challenge in regions with inadequate water and sanitation infrastructure.

The bacterium produces cholera toxin, leading to massive fluid secretion and the characteristic "rice-water" diarrhea that can cause severe dehydration and death within hours if untreated.

Transmission occurs primarily through the ingestion of water contaminated with fecal matter from infected individuals. The bacterium can also be transmitted through contaminated food (e.g., seafood harvested from contaminated waters).

Prevention and control measures include access to safe drinking water, improved sanitation, proper disposal of human waste, and oral rehydration therapy during outbreaks.

b) Tuberculosis

Tuberculosis (TB) is a leading global infectious disease caused by Mycobacterium tuberculosis, primarily affecting the lungs. *M. tuberculosis* has evolved sophisticated mechanisms to survive within macrophages, including inhibition of phagosome-lysosome fusion and resistance to oxidative stress.

Transmission occurs through the airborne route when individuals with active pulmonary TB release aerosols containing the bacteria during coughing, sneezing, or speaking. These aerosols can remain suspended in the air for extended periods, particularly in poorly ventilated spaces. After

inhalation, the bacteria establish infection in the lungs, where they may remain dormant as latent TB infection or progress to active disease.

Prevention strategies include vaccination with Bacille Calmette-Guérin (BCG), early diagnosis, isolation of infectious cases, and contact tracing. However, the emergence of multidrug-resistant TB and extensively drug-resistant TB presents challenges to control efforts. Addressing social determinants like poverty, overcrowding, and malnutrition is crucial for sustainable TB control.

3. The Role of Microorganisms in the Environment

Environmental microbiology is the study of microorganisms in their natural environments and their roles in ecological processes. Microorganisms are omnipresent in nature, found in virtually every habitat on Earth. They play critical roles in maintaining ecosystem balance through nutrient cycling, organic matter decomposition, and interactions with other organisms.

3.1. Microbial diversity in different environmental niches

Microorganisms exhibit remarkable diversity across different environmental niches, adapting to various conditions through specialized metabolic pathways and structural adaptations. Recent advances in metagenomics have revolutionized our understanding of microbial communities in diverse environments, allowing scientists to analyze complex microbial ecosystems without the need for traditional culturing methods.

3.1.1. Microbial communities and interactions

Microbial communities form complex networks of interactions including:

Symbiotic relationships with plants and animals

Competition for resources and ecological niches

Cooperative metabolic activities

Predator-prey relationships

3.1.2. Adaptation mechanisms for environmental survival

Microorganisms have developed various strategies to survive in changing environmental conditions, including the production of protective structures (spores, capsules), metabolic versatility to utilize different energy sources, biofilm formation for protection against environmental stressors, and horizontal gene transfer to acquire beneficial traits

The gut microbiome demonstrates adaptation through colonization resistance, where symbiotic bacteria produce bacteriocins, antimicrobial peptides, and other proteins that destroy pathogenic bacteria. In environmental contexts, similar mechanisms help microbial communities maintain stability despite changing conditions.

3.1.3. Extremophiles and their specialized adaptations

Extremophiles are microorganisms that thrive in environments considered hostile to most life forms, including thermophiles (hot springs and hydrothermal vents), psychrophiles (arctic and

antarctic regions), Halophiles (hypersaline environments), acidophiles and alkaliphiles (extreme pH conditions), and barophiles (high-pressure deep-sea environments). These organisms have evolved specialized adaptations such as unique membrane structures, protective enzymes, and metabolic pathways that allow them to not only survive but also thrive in conditions that would be lethal to most organisms.

3.2. Microorganisms in biogeochemical cycling

Microorganisms play crucial roles in global biogeochemical cycles, facilitating the transformation and movement of essential elements through the environment. Their metabolic activities are fundamental to ecosystem functioning and sustainability.

3.2.1. Carbon cycle

Microorganisms are key players in the global carbon cycle, facilitating carbon fixation, decomposition, and transformation processes (Figure 96). They contribute to:

- **Primary production**: Photosynthetic microorganisms, including cyanobacteria and algae, capture CO_2 from the atmosphere and convert it into organic carbon compounds through photosynthesis. This process represents a significant carbon sink.
- **Decomposition**: Heterotrophic microorganisms decompose organic matter, releasing CO_2 back into the atmosphere through respiration. This decomposition process is essential for nutrient recycling.
- **Carbon sequestration**: Microbes contribute to carbon storage in soils and sediments.
- **Methane cycling**: In anaerobic environments, specialized microorganisms called methanogens produce methane as a metabolic byproduct, while methanotrophs consume methane, preventing its release into the atmosphere. This microbial regulation of methane is crucial for climate stability, as methane is a potent greenhouse gas.

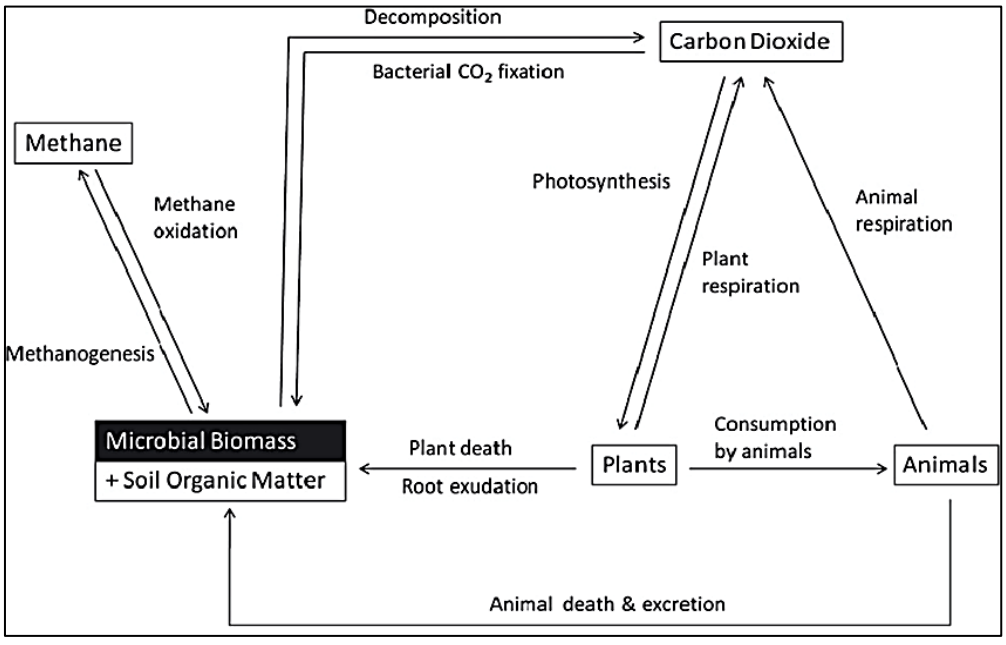


Figure 96. The carbon cycle.

3.2.2. Nitrogen cycle

The nitrogen cycle (Figure 97) is heavily dependent on microbial activities that transform nitrogen between its various forms:

- **Nitrogen fixation:** Nitrogen-fixing bacteria convert atmospheric nitrogen (N_2) into ammonia (NH_3) or ammonium (NH_4^+), forms usable by plants and other organisms. Examples include *Rhizobium* species, which form symbiotic relationships with leguminous plants, while free-living bacteria like Azotobacter can fix nitrogen independently.
- **Ammonification:** Decomposer microorganisms (bacteria and fungi) convert organic nitrogen in dead organisms and waste materials to (NH_3).
- **Nitrification:** A two-step process where ammonia-oxidizing bacteria (e.g., *Nitrosomonas*) convert ammonia to nitrite (NO_2^-), and nitrite-oxidizing bacteria (e.g., *Nitrobacter*) further oxidize nitrite to nitrate (NO_3^-). These bacteria are named nitrifying bacteria.
- **Denitrification:** Anaerobic bacteria (e.g., *Pseudomonas, Bacillus*) reduce (NO_3^-) to nitrogen gas (N_2), completing the cycle by returning nitrogen to the atmosphere.

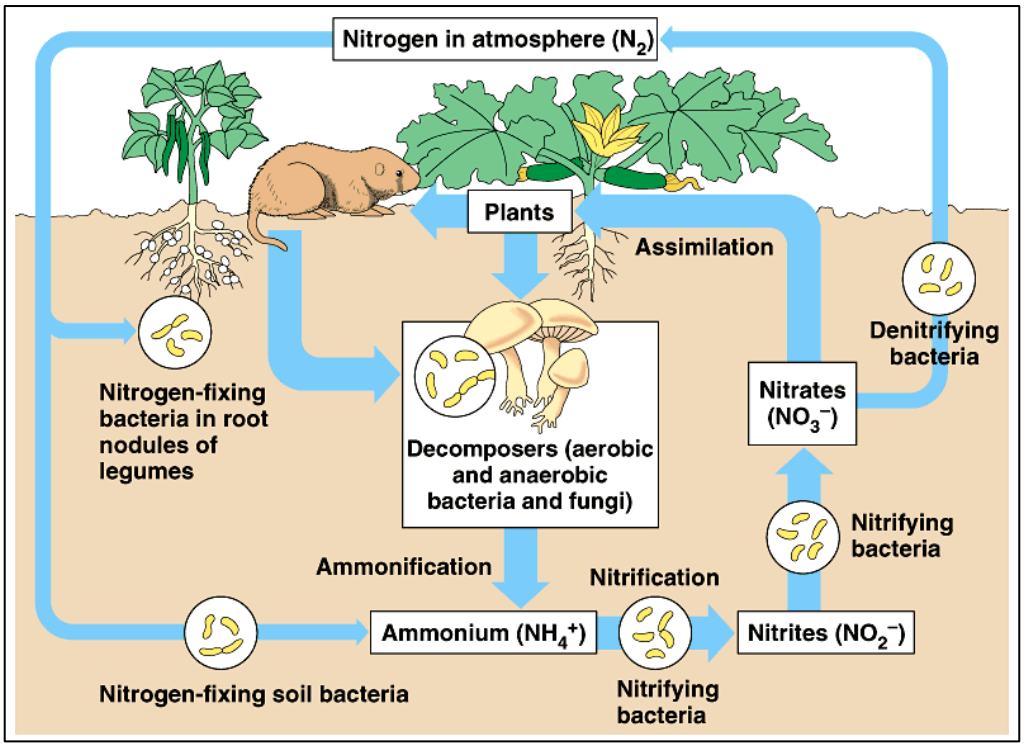


Figure 97. The nitrogen cycle.

These microbial processes are essential for soil fertility and plant growth, making microorganisms crucial for agricultural productivity and ecosystem health. The nitrogen cycle also has significant environmental implications, as imbalances can lead to issues such as eutrophication of water bodies and the release of nitrous oxide, a potent greenhouse gas.

3.2.3. Other Nutrient Cycles

Microorganisms facilitate numerous other nutrient transformations:

- **Sulfur cycle:** Microbial oxidation and reduction of sulfur compounds.
- **Phosphorus cycle:** Microbes solubilize phosphate, making it available for plant uptake.

In the sulfur cycle, microorganisms oxidize hydrogen sulfide to sulfate or reduce sulfate to hydrogen sulfide, depending on environmental conditions. These transformations are crucial in both terrestrial and aquatic ecosystems, particularly in anaerobic environments like wetlands and marine sediments.

Phosphorus, an essential nutrient for all living organisms, is often a limiting factor in ecosystems. Microorganisms play a vital role in making phosphorus available to plants by solubilizing inorganic phosphate and mineralizing organic phosphorus compounds.

3.3. Microorganisms in different ecosystems

3.1 Soil microbiology

Soil serves as one of the richest microbial habitats on Earth. Microorganisms in soil:

- ✓ Improve soil structure and fertility through organic matter decomposition.
- ✓ Enhance mineral absorption for plants.
- ✓ Fix nitrogen and make other nutrients available.
- ✓ Remediate soil contaminants through bioremediation.
- ✓ Produce hormones that stimulate plant growth.

The rhizosphere, the narrow region of soil directly influenced by root secretions, hosts diverse microbial communities that form intimate associations with plants. These plant-microbe interactions can be beneficial (promoting growth), neutral, or pathogenic.

3.2 Aquatic microbiology

Aquatic environments host diverse microbial communities that:

- ✓ Form the base of aquatic food webs through primary production.
- ✓ Decompose organic matter in water bodies.
- ✓ Cycle nutrients in freshwater and marine ecosystems.
- ✓ Contribute to water purification processes.

Marine microorganisms play particularly important roles in global carbon cycling and climate regulation through their involvement in the biological pump that sequesters carbon in deep ocean sediments.

3.3 Atmospheric microbiology

The atmosphere contains numerous microorganisms that:

- ✓ Disperse across geographical barriers via air currents.
- ✓ Contribute to cloud formation and precipitation.

- ✓ Form bioaerosols that can impact air quality.
- ✓ Participate in atmospheric chemical transformations.

3.4. Microorganisms in environmental applications

3.4.1. Bioremediation

Microorganisms serve as effective agents for environmental remediation:

- ✓ Biodegradation of organic pollutants like petroleum hydrocarbons.
- ✓ Transformation of heavy metals into less toxic forms.
- ✓ Degradation of pesticides and other xenobiotics.
- ✓ Soil bioremediation to restore contaminated areas.

Bioremediation utilizes the inherent metabolic capabilities of microorganisms to degrade or transform environmental pollutants into less harmful substances. This strategy is often more cost-effective and environmentally friendly than physical or chemical remediation methods.

Different bioremediation strategies include in situ treatments, where contaminants are treated at the site of contamination, and ex situ treatments, which involves the removal of contaminated material for treatment at a different location. The efficacy of bioremediation depends on various factors, including the nature of the contaminant, environmental conditions, and the presence of suitable microorganisms.

Recent advances in genetic engineering and synthetic biology have expanded the potential applications of bioremediation, with the development of genetically modified microorganisms designed to degrade specific pollutants more efficiently.

3.4.2. Agricultural Applications

In agriculture, microorganisms provide sustainable alternatives to chemical inputs:

- Biological nitrogen fixation reduces the need for synthetic fertilizers.
- Plant growth-promoting microorganisms enhance crop yields.
- Microbial biopesticides offer alternatives to chemical pest control.
- Mycorrhizal fungi improve plant nutrient uptake.

The integrated approach of using microbes along with organic growth enhancers can revolutionize sustainable agriculture practices. As awareness grows about the adverse effects of chemical fertilizers and pesticides on soil and the environment, there is a global shift toward organic farming approaches that leverage beneficial microorganisms for sustainable soil health and human health benefits.

3.4.3. Waste management

Microorganisms are essential for effective waste management. Composting processes rely on microbial decomposition. Bacteria, fungi, and actinomycetes which decompose organic waste materials under aerobic conditions, producing a nutrient-rich soil amendment.

Wastewater treatment plants utilize microbial communities to break down organic matter. This process relies on complex microbial communities to remove organic matter, nutrients, and pathogens from sewage and industrial effluents.

Anaerobic digestion by microbes produces biogas from organic waste. is increasingly used for the treatment of organic waste, producing biogas (a mixture of methane and carbon dioxide) that can be used as a renewable energy source.

Chapter VIII

Virology

Chapter VIII. Virology

Virology is the science discipline dedicated to the study of viruses, which are a strong driving force of life and evolution. As the most abundant biological entities on Earth, viruses occupy almost every ecosystem and infect all types of life forms.

This chapter explores the fundamental characteristics of viruses, including their structure, replication strategies, and classification. It also emphasises three major categories: bacteriophages (viruses that infect bacteria), plant viruses (which cause numerous economically important crop diseases), and animal viruses (including those that affect humans and livestock).

1. Introduction

1.1. Historical view of virology

Early Observations and Foundations (Pre-1900)

The history of viral diseases dates back to ancient civilizations, with evidence found on Egyptian temple walls showing polio (1580-1350 BCE) and smallpox lesions on the mummy of Ramesses V (1143 BCE). Though viruses weren't yet understood, their effects were documented for millennia.

Key developments:

- **1796:** Edward Jenner pioneers smallpox vaccination using cowpox material.
- **1885:** Louis Pasteur develops the first rabies vaccine.
- **1892:** Dmitri Ivanovsky demonstrates tobacco mosaic disease agent can pass through bacteria-proof filters.
- **1898:** Martinus Beijerinck coins the term "virus" and establishes a conceptual foundation for virology
- **1898:** Friedrich Loeffler and Paul Frosch isolate the foot-and-mouth disease virus, marking the beginning of animal virology.

Microbiology period (1898-1934)

During this period, ultrafiltration technology enabled significant advances in virus isolation and study. Scientists began to understand viruses as distinct from bacteria, though their true nature remained unclear**.**

Key developments:

- **1901:** Walter Reed identifies yellow fever as the first human viral disease transmitted by mosquitoes.
- **1911:** Francis Rous discovers Rous sarcoma virus, the first known retrovirus.
- **1917:** Felix d'Herelle discovers bacteriophages.

- **1931:** Ernest Goodpasture develops a method to grow viruses in embryonated chicken eggs.
- **1933: The** First human influenza A virus is isolated by Richard Shope.

Biochemistry Era (1935-1954)

This period marked a turning point in understanding the molecular nature of viruses. Wendell Stanley's crystallization of the tobacco mosaic virus (TMV) in 1935 demonstrated that viruses had properties of both living and non-living matter.

Significant advances:

- **1935:** Wendell Stanley crystallizes TMV, earning the 1946 Nobel Prize.
- **1939:** First visualization of viruses using electron microscopy.
- **1952:** Alfred Hershey and Martha Chase prove that DNA is genetic material in bacteriophages.
- **1954:** John Enders, Thomas Weller, and Frederick Robbins develop methods to grow poliovirus in tissue culture.

Genetics Era (1955-1984)

This period was characterized by breakthroughs in understanding viral genetics and replication mechanisms. The discovery of reverse transcriptase challenged the central dogma of molecular biology.

Major acheivements:

- **1957:** Howard Temin discovers Rous sarcoma virus can transform cells.
- **1970:** Howard Temin and David Baltimore discover reverse transcriptase.
- **1977:** Frederick Sanger sequences first complete viral genome (bacteriophage φX174).
- **1983:** Françoise Sinoussi, Luc Montagnier, and Robert Gallo discover HIV-1.

Molecular Biology Era (1985- Present)

The modern era has witnessed the remarkable integration of virology with molecular biology and genetic engineering, leading to revolutionary advances in vaccine development and antiviral treatments.

Recent advancements:

- **1985:** George Smith introduces phage display technology.
- **1986:** First genetically engineered hepatitis B vaccine approved.
- **1996:** Highly active antiretroviral therapy (HAART) introduced for HIV treatment.
- **2003:** SARS coronavirus identified.
- **2020:** mRNA vaccines developed for COVID-19 in record time.

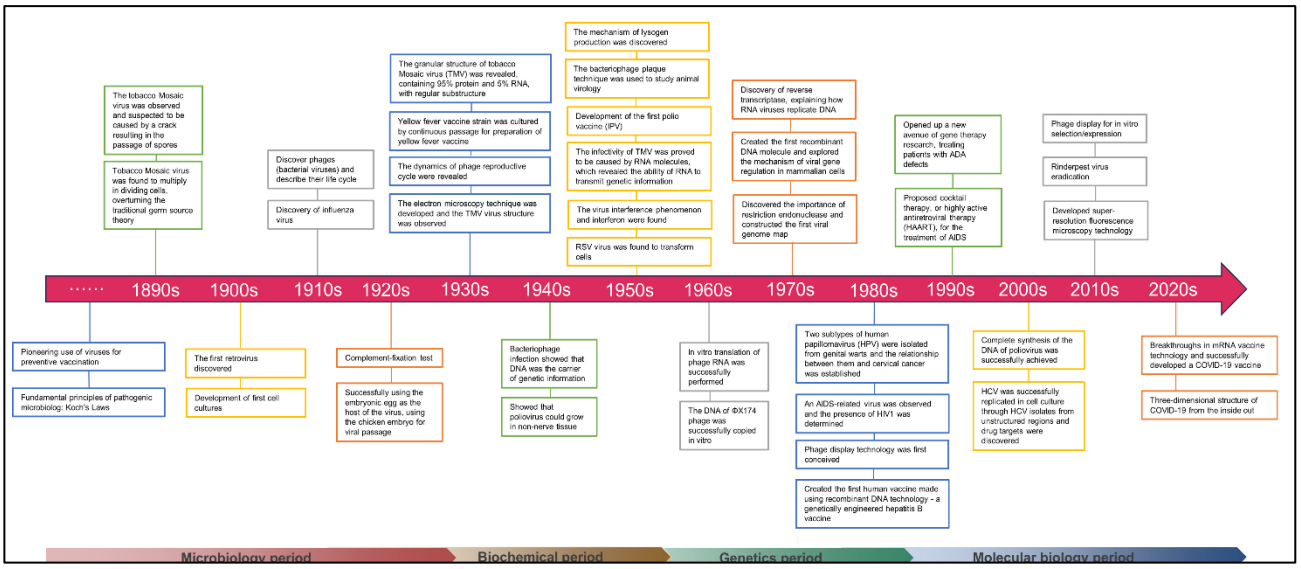


Figure 98. History of virology from the late 19th century to the 2020s.

1.2. General characteristics of viruses

Viruses are obligatory intracellular parasites that can only replicate inside living cells. They are extremely small, ranging from 20-500 nanometers (nm) in diameter, making them invisible under ordinary light microscopes. Unlike cellular organisms, viruses contain only one type of nucleic acid (either DNA or RNA), but never both, enclosed within a protein coat called a capsid.

1.2.1. Molecular structure and composition

The basic structure of a virus includes:

a) Genome or nucleic acid

The viral genome can be DNA or RNA (but never both), single or double-stranded, linear or circular, and segmented or non-segmented. A virus that contains the DNA protein is called a DNA Virus, and that which contains RNA is called an RNA virus. RNA viruses can have positive-sense, negative-sense, or ambisense genomes.

b) Capsid

The capsid is the protective protein coat. It is composed of protein subunits called capsomeres that protect the viral genome. Capsomers assemble into specific geometric patterns. Based on the arrangement of these capsomeres, virus capsids exhibit different symmetry structures (Figure 99):

- **Helical (spiral) symmetry:** Capsomers interact with each other and with the nucleic acid to form a spiral tube (Figure 99a). Most of these viruses are enveloped, and all of them contain RNA proteins. The helix may be rigid (like tobacco mosaic virus) or flexible (like paramyxoviruses)
- **Icosahedral symmetry:** Capsomers arranged in a polyhedron with 20 triangular faces, 30 edges, and 12 vertices. This symmetry is common in human pathogens, e.g., adenoviruses and herpes viruses.
- **Complex symmetry:** Irregular arrangements found in poxviruses.

- **Binal symmetry:** This is a type of complex symmetry where the virus structures have heads and tails, as seen in bacteriophages.

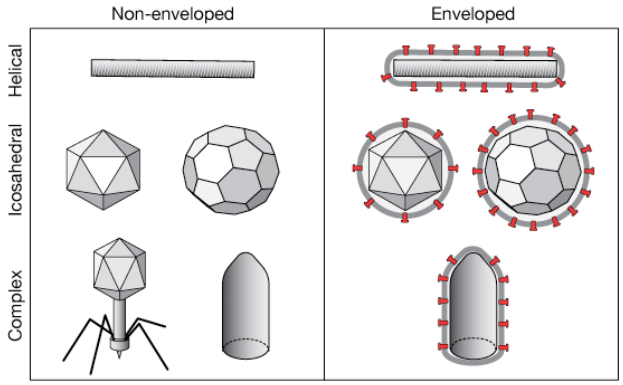


Figure 99. The main symmetry structures of viruses (Helical, icosahedral, and complex).

c) Envelope: Present in some viruses, surrounding the nucleocapsid. Its lipid bilayer is obtained by budding through host cell membranes. The envelope also contains viral glycoproteins.

1.2.2. Viral replication cycle

The viral replication cycle occurs in host cells and consists of several distinct stages:

a) Attachment (Absorption)

The virus binds to specific receptors on the host cell surface through viral surface proteins. This specificity determines which cells and organisms a virus can infect. Host cells devoid of the appropriate receptors are not infected by the virus.

b) Penetration (Entry)

After attachment, the virus particle enters the cell through two main mechanisms:

- For enveloped viruses: Fusion of viral envelope with cell membrane or endocytosis (Figure 100).

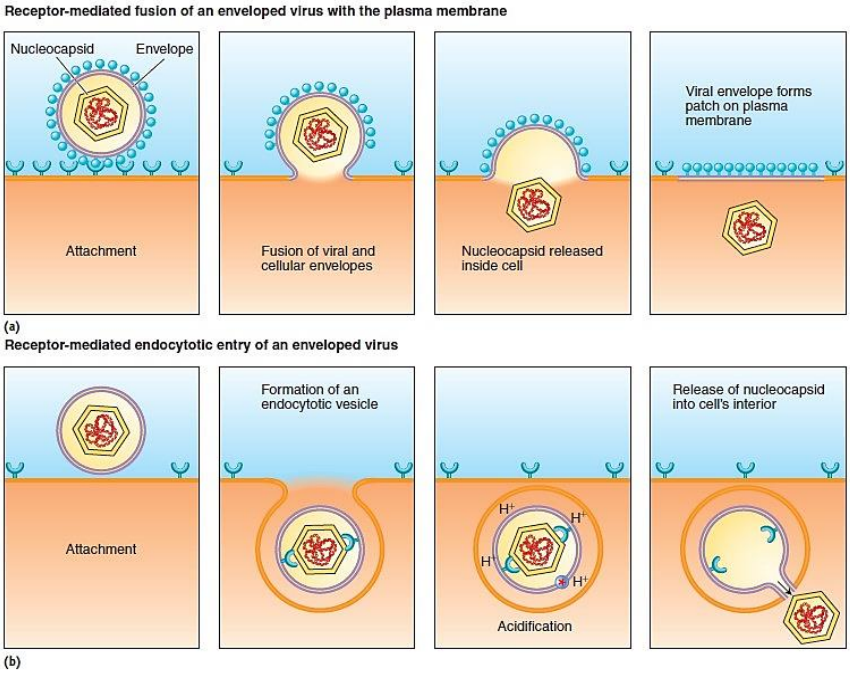


Figure 100. Entry for enveloped viruses. **(a):** Viral entry steps by fusion; **(b):** viral entry steps by endocytosis.

- For naked viruses: Typically, through receptor-mediated endocytosis (Figure 101).

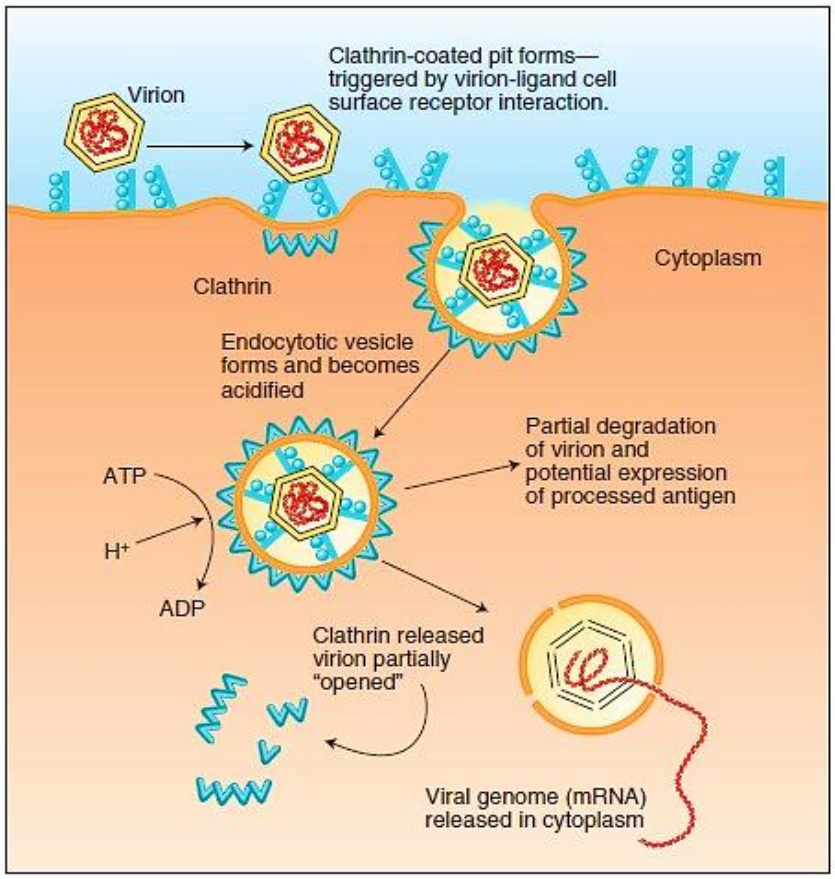


Figure 101. Entry for naked viruses.

c) Uncoating

The viral capsid is removed, releasing the nucleic acid into the host cell cytoplasm. This step is crucial for initiating viral replication.

d) Replication and protein synthesis

The viral genome is replicated, and viral proteins are synthesized using host cell machinery. The specific strategy depends on the type of viral genome:

- DNA viruses: Generally, replicate in the nucleus using host enzymes.
- RNA viruses: Often replicate in the cytoplasm using viral RNA-dependent RNA polymerases.

e) Assembly

Newly synthesized viral components assemble to form new virus particles (virions). This process occurs at specific sites within the infected cell and involves the condensation of protein capsids around viral nucleic acid molecules.

f) Maturation

During maturation, the assembled virus particles undergo final processing steps to become infectious. This often involves proteolytic cleavage of viral proteins.

f) Release

The final step is the release of mature virions from the host cell through:

- Lysis: Naked viruses often cause rupture of the host cell membrane
- Budding: Enveloped viruses typically exit by budding through the cell membrane without necessarily killing the cell.

1.2.3. Viral taxonomy and classification

1.2.3.1. Early classification approaches

a) Clinical and ecological classification

Key classification criteria include:

- Disease manifestation (respiratory, nervous, digestive viruses).
- Organ tropism (liver, lung, brain, intestine viruses).
- Transmission patterns (arthropod-borne, airborne, vertical, venereal).

Limitation Example: Hepatitis viruses (A-E) cause similar diseases but belong to different families (Picornaviridae, Hepadnaviridae, Flaviviridae, Deltaviridae, Caliciviridae).

b) Physicochemical and antigenic classification

Key classification criteria include:

- Virion size (determined by ultrafiltration, ultracentrifugation, and electron microscopy)
- Virion morphology (observed via electron microscopy)
- Virion stability (under varying pH, temperature, lipid solvents, detergents, radiation)
- Virus antigenicity (determined through serological tests).

1.2.3.2. Modern classification system

a) Universal system for virus taxonomy (USVT)

The International Committee on Taxonomy of Viruses (ICTV) established this comprehensive system based on multiple viral characteristics.

Key classification criteria include:

- Morphological properties: Virus size and shape, capsid symmetry (helical, icosahedral, complex), envelope presence or absence.
- Genomic properties: Nucleic acid type (DNA or RNA), strandedness (single-stranded or double-stranded), sense (positive or negative), and genome organization (segmented/non-segmented, linear/circular).
- Replication strategies.
- Phylogenetic relationships.

The ICTV has established a hierarchical system for classifying viruses using standardized suffixes to denote different taxonomic ranks (Table 10).

Table 10. Taxonomic Hierarchy in virus classification

Taxonomic rank	Suffix	Example
Realm	-viria	Riboviria
Kingdom	-virae	Orthornavirae
Phylum	-viricota	Pisuviricota
Class	-viricetes	Pisoniviricetes
Order	-virales	Mononegavirales
Family	-viridae	Poxviridae
Subfamily	-virinae	Chordopoxvirinae
Genus	-virus	Capripoxvirus
Species	No specific suffix	Variola virus

b) Baltimore classification system

Developed by Nobel laureate David Baltimore, this system classifies viruses based on their method of mRNA production during replication. It divides viruses into seven classes:

- **Class I:** Double-stranded DNA viruses (dsDNA viruses), e.g., Herpesviridae and Poxviridae.
- **Class II:** Single-stranded DNA viruses (ssDNA viruses), e.g., Parvoviridae.
- **Class III:** Double-stranded RNA viruses (dsRNA viruses), e.g., Reoviridae.
- **Class IV:** Positive-sense single-stranded RNA viruses ((+)ssRNA viruses), e.g., Picornaviridae and Flaviviridae.
- **Class V:** Negative-sense single-stranded RNA viruses ((-)dsRNA viruses), e.g., Orthomyxoviridae and Rhabdoviridae.
- **Class VI:** RNA retroviruses with DNA intermediate (ssRNA-RT viruses), e.g., Retroviridae.
- **Class VII:** DNA viruses with RNA intermediate (dsDNA-RT viruses), e.g., Hepadnaviridae.

c) Phylogenetic classification

Modern virus taxonomy increasingly incorporates genetic sequence analysis to establish evolutionary relationships.

d) Classification of major virus groups

- **DNA viruses:** Table (11) represents the classification of major DNA virus groups.

Table 11. Classification and characteristics of major DNA virus families.

Family	Genome type	Envelope	Capsid symmetry	Notable examples
Poxviridae	dsDNA, linear	Yes	Complex	Variola virus, Vaccinia virus
Herpesviridae	dsDNA, linear	Yes	Icosahedral	HSV-1, HSV-2, VZV, EBV, CMV
Adenoviridae	dsDNA, linear	No	Icosahedral	Human adenovirus
Papillomaviridae	dsDNA, circular	No	Icosahedral	HPV
Polyomaviridae	dsDNA, circular	No	Icosahedral	JC virus, BK virus

Table 11. Continued.

Family	Genome type	Envelope	Capsid symmetry	Notable examples
Hepadnaviridae	dsDNA, partially circular	Yes	Icosahedral	Hepatitis B virus
Parvoviridae	ssDNA, linear	No	Icosahedral	B19 virus

- **RNA viruses:** Table (12) represents the classification of major RNA virus groups.

Table 12. Classification and characteristics of major RNA virus families.

Family	Genome type	Envelope	Capsid symmetry	Segmentations	Notable examples
Reoviridae	dsRNA	No	Icosahedral	Segmented	Rotavirus, Orbivirus
Picornaviridae	ssRNA(+)	No	Icosahedral	Non-segmented	Poliovirus, Rhinovirus, HAV
Caliciviridae	ssRNA(+)	No	Icosahedral	Non-segmented	Norovirus, HEV
Flaviviridae	ssRNA(+)	Yes	Icosahedral	Non-segmented	HCV, Yellow fever virus, Dengue virus
Togaviridae	ssRNA(+)	Yes	Icosahedral	Non-segmented	Rubella virus, Alphavirus
Coronaviridae	ssRNA(+)	Yes	Helical	Non-segmented	SARS-CoV-2, MERS-CoV
Orthomyxoviridae	ssRNA(-)	Yes	Helical	Segmented	Influenza virus
Paramyxoviridae	ssRNA(-)	Yes	Helical	Non-segmented	Measles virus, Mumps virus, RSV
Rhabdoviridae	ssRNA(-)	Yes	Helical	Non-segmented	Rabies virus
Filoviridae	ssRNA(-)	Yes	Filamentous	Non-segmented	Ebola virus, Marburg virus
Bunyaviridae	ssRNA(-)	Yes	Helical	Segmented	Hantavirus
Retroviridae	ssRNA(+) with RT	Yes	Icosahedral	Non-segmented	HIV, HTLV

2. Bacterial Viruses (Bacteriophages)

Bacteriophages, commonly referred to as phages, are viruses that specifically infect and replicate within bacterial cells. The term "bacteriophage" is derived from "bacteria" and the Greek word *phagein*, meaning "to devour," highlighting their ability to destroy bacterial hosts during their replication cycle. Bacteriophages are obligate parasites, relying entirely on bacterial cells for reproduction. Infection with bacteriophages is typically restricted to specific strains within a single bacterial species, showcasing their host specificity.

2.1. Structure of bacteriophages

Bacteriophages, or phages, exhibit diverse structural forms, but most share a common architecture comprising a head (capsid), tail, and baseplate. Their structure is optimized for infecting bacterial cells (Figure 102).

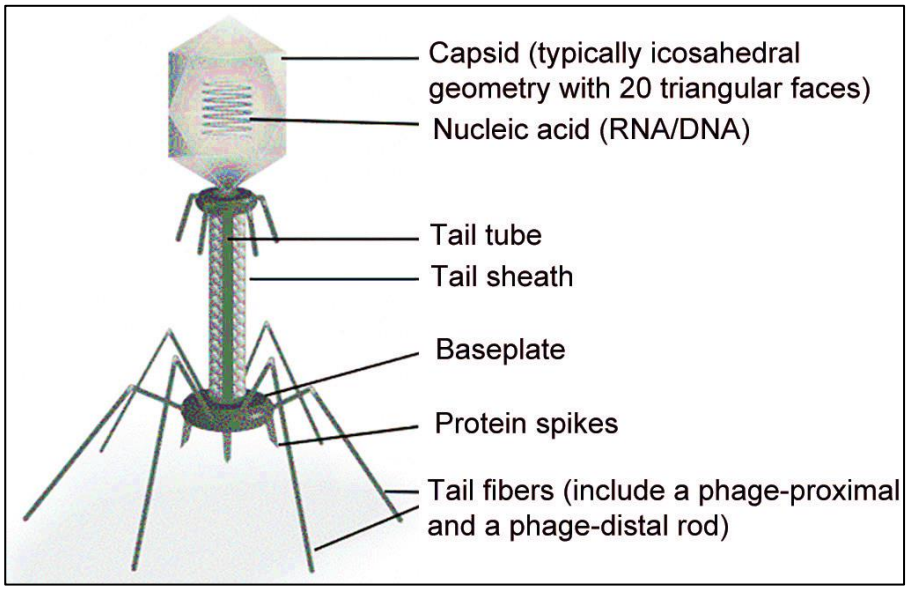


Figure 102. Diagrammatic representation of a bacteriophage.

- **Head (Capsid):** The capsid is an icosahedral or filamentous protein shell that encloses the viral genome. It is composed of protein subunits called capsomeres. The capsid protects the nucleic acid and facilitates its delivery into the host cell.
- **Tail:** The tail is a hollow tube that connects the head to the baseplate. It serves as a conduit for transferring genetic material into the bacterial host. Tails can be contractile (as in Myoviridae), long and non-contractile (Siphoviridae), or short (Podoviridae).
- **Baseplate and Tail Fibers:** The baseplate anchors the phage to the bacterial surface, while tail fibers aid in host recognition and attachment. These structures are critical for specificity in infection.
- **Lipid Envelope (Rare):** Some phages, such as those in the Cystoviridae family, possess a lipid envelope derived from the host cell membrane. This envelope contains membrane proteins facilitating attachment and entry into bacterial cells.

Phages range in size from 24 nm to 200 nm, with T4 phages being among the largest, measuring approximately 200 nm in length.

2.2. Classification of bacteriophages

Bacteriophages are classified based on their morphology, nucleic acid type, and life cycle. The International Committee on Taxonomy of Viruses (ICTV) governs phage classification.

2.2.1. Morphological classification

Bradley's classification divides bacteriophages into distinct fundamental morphological types, as illustrated in Table (13).

Table 13. Classification of bacteriophages.

Shape	Nucleic acid	Family	Example
Tailed	dsDNA	*Myoviridae* (Tail contractile)	T4
		Siphoviridae (Tail long, non-contractile)	λ
		Podoviridae (Tail short)	T7
Polyhedral (Icosahedral capsids with varying nucleic acids)	ssDNA	*Microviridaae*	φX174
	dsDNA	*Corticoviridae, Tectiviridae,* SH1, STIV	PM2, PRD1
	ssRNA	*Leviviridae*	MS2
	dsRNA	*Cystoviridae*	φ6
Filamentous (Long filament-like structures)	ssDNA	*Inoviridae*	MI3
	dsDNA	*Lipothrixviridae, Rudiviridae*	TTV1, SIRV-1
Pleomorphic (Irregular shapes)	dsDNA	*Plasmaviridae, Fuselloviridae, Guttaviridae, Ampullaviridae, Bicaudaviridae, Globuloviridae*	L2, SSV-1, His-1

2.2.2. Genomic classification

Bacteriophage genomes are highly diverse:

- Most common: Double-stranded DNA (dsDNA).
- Less common: Single-stranded DNA (ssDNA), single-stranded RNA (ssRNA), and double-stranded RNA (dsRNA).

2.2.3. Life Cycle-based classification

Phages are categorized based on their replication strategies:

- **Virulent phages:** Exhibit lytic cycles only, killing the host bacteria after replication.
- **Temperate phages:** Can alternate between lytic and lysogenic cycles, integrating their genome into the host DNA as prophage during lysogeny.

2.3. Life cycle of bacteriophages

Bacteriophages exhibit varying life cycles based on their interaction with the host bacteria. These cycles include the lytic cycle and the lysogenic cycle.

2.3.1. Lytic cycle (Virulent phages)

The lytic cycle is characteristic of virulent phages, which replicate rapidly within the host and cause its destruction (Figure 103). The steps are as follows:

a) Attachment

The phage attaches to specific receptors on the bacterial surface, such as lipopolysaccharides, teichoic acids, or proteins. This specificity ensures that phages infect only compatible hosts.

b) Penetration

After attachment, the phage injects its genetic material into the bacterial cytoplasm. Tailed phages like Myoviridae use a syringe-like tail contraction mechanism, while others like Podoviridae enzymatically degrade portions of the bacterial membrane to facilitate DNA entry.

c) Replication (Synthesis)

Inside the host cell, the phage genome hijacks bacterial machinery to produce viral components:
Early genes encode enzymes to degrade host DNA and modify bacterial RNA polymerase.
Late genes direct the synthesis of structural proteins for new virions.

d) Assembly

Newly synthesized viral components are assembled into complete virions. This process involves the packaging of genetic material into capsids and the attachment of tails and other structures.

e) Lysis and release

Endolysins and holins degrade the bacterial cell wall, causing cell lysis and releasing progeny phages into the environment to infect new hosts.

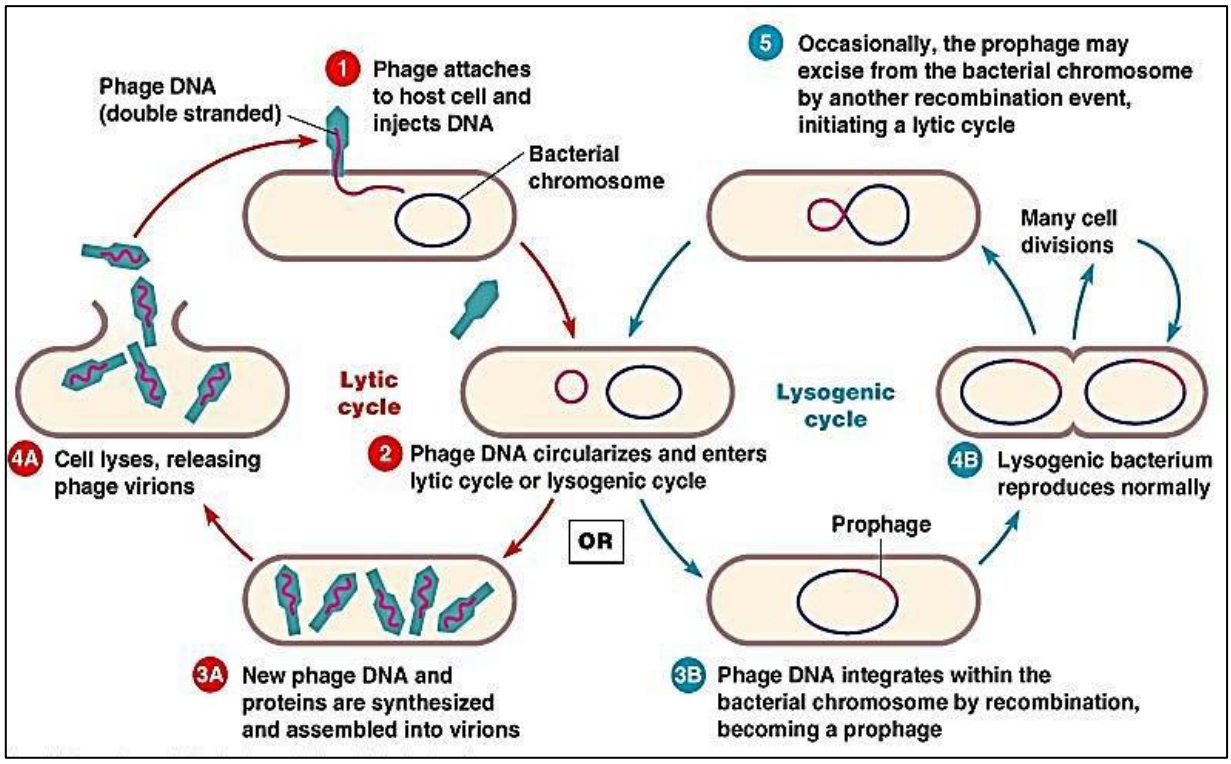


Figure 103. Lytic and lysogenic cycles of bacteriophages.

2.2.2. Lysogenic cycle (Temperate phages)

Temperate phages can alternate between lytic and lysogenic cycles. In lysogeny, they integrate their genome into the host's chromosome as a prophage without killing the host immediately (Figure 102).

a) Attachment and penetration

Similar to the lytic cycle, temperate phages attach to specific receptors and inject their DNA into the host cell.

b) Integration

The phage genome integrates into the bacterial chromosome using site-specific recombination mechanisms. The integrated genome is referred to as a prophage.

c) Dormancy (Lysogeny)

The prophage remains dormant, replicating passively along with the host genome during cell division. During this phase:

- ✓ The host bacteria is termed a lysogen.
- ✓ The prophage may confer beneficial traits to its host through lysogenic conversion, such as increased pathogenicity or antibiotic resistance.

d) Induction

Environmental stressors (e.g., UV radiation) can trigger induction, where the prophage excises itself from the bacterial genome and enters the lytic cycle.

2.4. Applications: Phage therapy

Phage therapy is the therapeutic management of bacterial infections with phages (bacteriophages). The approach, which has gained a renewed interest due to the rising prevalence of diseases caused by multidrug-resistant bacteria, possesses significant advantages.

The key advantages over antibiotics include

- **Specificity**: Target specific bacterial strains without affecting beneficial microflora.
- **Effectiveness**: Bactericidal action with multiplication at the infection site.
- **Production**: Simpler and less expensive than antibiotics.
- **Safety**: Well-tolerated with no documented serious side effects.
- **Adaptability**: New phages can be isolated within days when resistance develops.

The clinical applications of phage therapy may include:

- Treatment of multidrug-resistant bacterial infections.
- Applicable in various environments: human, animal, food safety, biofilm control.
- Effective against both Gram-positive and Gram-negative bacteria.

However, some limitations of this approach can be highlighted, such as ineffectiveness against intracellular bacteria, potential neutralizing antibody development in recurrent infections, and the need for specialized training for healthcare providers.

2. Plant viruses

Plant viruses are obligate intracellular parasites that depend entirely on host cellular machinery for their replication and spread. These submicroscopic pathogens consist of a nucleic acid genome

encapsidated in a protein coat, and they cause numerous economically important diseases in crops worldwide. Most plant viruses infect a limited range of plant species, though some have a wider host range. They cause chronic degenerative diseases that decrease plant fitness, resulting in significant agricultural losses.

2.1. Structure of Plant Viruses

Plant virus particles (virions) are composed of two main components: capsid and genome.

2.1.1. Capsid structure

Based on the arrangement of capsomers of the protein capsid, plant viruses are categorized into two basic shapes:

- **Helical:** Capsomers arranged in a helical pattern (e.g., Tobacco mosaic virus).
- **Icosahedral:** Capsomers form triangles arranged in a polyhedron (usually an icosahedron with twenty sides).

2.1.2. Genome organization

Plant viruses are non-cellular entities composed of nucleic acid (DNA or RNA) enclosed in a protein capsid without a membrane envelope. The nucleic acid genome may be ssDNA, dsDNA, ssRNA (either positive-sense or negative-sense), or dsRNA.

2.2. Replication of plant viruses

The replication process varies depending on the type of viral genome but generally follows these stages:

2.2.1. Attachment and entry

Viruses enter plant cells through wounds created artificially by vectors (Figure 104), or naturally when infected pollen deposits inside an ovule. Unlike animal viruses, plant viruses cannot actively penetrate intact cell walls.

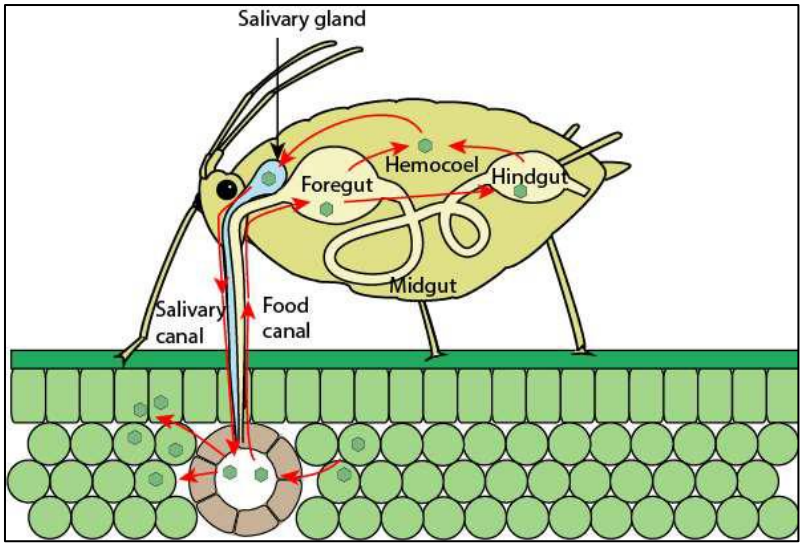


Figure 104. Penetration of plant viruses by vectors.

2.2.2. Uncoating

Once inside the host cell, the virus sheds its protein coat (capsid), releasing its genome to become accessible to enzymes required for replication.

2.2.3. Genome expression and replication

The replication strategy depends on the type of viral genome:

a) DNA viruses

- **dsDNA viruses:** Transcription occurs by host RNA polymerase II from dsDNA. Some dsDNA viruses replicate through RNA intermediates using reverse transcription.
- **ssDNA viruses (e.g., Geminiviridae):** Replication occurs through a dsDNA intermediate using host DNA polymerase.

b) RNA viruses

- **dsRNA viruses (e.g., Phytoreoviridae):** Contain viral RNA-dependent RNA polymerase (RdRp) that transcribes the genome in the cytoplasm, often in specialized structures called viroplasms.
- **Positive-sense ssRNA viruses (most common in plants):** The genome directly functions as mRNA. After the translation of viral proteins, including RNA polymerase, the genome is replicated through a negative-strand intermediate.
- **Negative-sense ssRNA viruses (e.g., Rhabdoviridae):** Require packaged RNA-dependent RNA polymerase to synthesize positive-strand RNA from the negative-strand template. The positive strand then serves as mRNA for translation and as a template for replication.
- **ssRNA(RT) viruses:** Use reverse transcription to first make dsDNA, which then undergoes transcription and translation.

2.2.4. Assembly

Once viral nucleic acid and protein subunits are synthesized, the virus assembles into complete virions. For RNA viruses, this typically occurs in the cytoplasm, while some DNA viruses assemble in the nucleus.

2.2.5. Movement and spread

Plant viruses encode specialized movement proteins that facilitate:

- Cell-to-cell movement through plasmodesmata (by increasing size exclusion limits).
- Long-distance movement through the phloem vascular system.

2.3. Classification of plant viruses

The Baltimore classification system classifies viruses based on their method of mRNA production in seven classes: Class I to Class VII (See the previous section of Virus taxonomy and classification).

Plant viruses are distributed across 17 families and 80 genera, with most being RNA viruses, particularly (+)ssRNA viruses (65.3% of plant virus species).

2.4. Transmission of plant viruses

Plant viruses use various mechanisms for transmission between hosts:

2.4.1. Vector-mediated transmission

Many plant viruses rely on biological vectors for transmission:

- Insects (especially aphids, leafhoppers, whiteflies): Transmission may be:
  - ✓ Non-persistent: The virus adsorbs to mouthparts (stylets) and remains infectious for minutes to hours.
  - ✓ Circulative: The virus circulates in the insect body with infectiousness lasting days.
  - ✓ Propagative: The virus replicates in the vector, providing lifelong infectiousness.
- Nematodes.
- Fungi.

2.4.2. Other transmission routes

Other transmission routes include:

- Mechanical transmission through wounds or direct contact.
- Seed and pollen transmission.
- Vegetative propagation.
- Parasitic plants (e.g., Cuscuta).
- Grafting and root coalescence.

2.5. Symptoms and impact on agriculture

Plant viruses cause various symptoms that significantly impact agricultural productivity:

2.5.1. Common symptoms

Common symptoms caused by plant viruses include:

- **Systemic symptoms:** Abnormal growth and developmental malformations (reduced growth, dwarfing, stunting), reduced lifespan or plant death, foliage symptoms (mosaics, yellowing, and ring spots), and symptoms on stems, fruits, and roots.
- **Local symptoms:** Necrotic lesions.

2.5.2. Agricultural impact

Plant viruses cause tremendous losses in crop production and quality worldwide. The economic impact stems from reduced yield, lower-quality produce, increased production costs for control measures, and trade restrictions due to quarantine regulations.

3. Animal Viruses

Viruses that infect animals are called animal viruses. They are included in the group Zoophaginae. Animal viruses contain DNA or RNA surrounded by a capsid, and some possess an additional lipid envelope. Animal viruses infect a wide range of vertebrate hosts and can cause various diseases, from mild respiratory infections to severe systemic diseases. Some of the examples

are poliovirus, yellow fever virus, vaccinia virus, adenovirus, rabies virus, mumps virus, measles virus, influenza virus, herpes virus, reovirus, dengue virus, and human immunodeficiency virus (HIV).

3.1. Structure and composition of animal viruses

Animal viruses are composed of:

- **Nucleic acid**: DNA or RNA (single or double-stranded) that encodes viral proteins
- **Capsid**: Arranged in either icosahedral symmetry (e.g., Herpesviridae, Picornaviridae), or helical symmetry (e.g., Rhabdoviridae, Paramyxoviridae).
- **Envelope**: Some animal viruses possess an outer lipid bilayer derived from host cell membranes with embedded viral glycoproteins (e.g., Herpesviridae, Paramyxoviridae).

The size of animal viruses ranges from approximately 20-30 nm (Picornaviridae) to 200-450 nm (Poxviridae).

3.2. Replication

The replication cycle of animal viruses follows several key steps: attachment (binding host cell receptors), penetration (via endocytosis, fusion, and translocation across the cell membrane), uncoating, biosynthesis, assembly, and release (via cell lysis, budding, and exocytosis).

3.3. Classification of animal viruses

Table (14) represents the classification of major animal virus groups.

Table 14. Main animal virus families and their characteristics.

Virus family	Nucleic acid	Structure	Example viruses/Diseases
Herpesviridae	dsDNA	Enveloped, icosahedral	Infectious bovine rhinotracheitis, Equine herpesvirus, Feline viral rhinotracheitis
Poxviridae	dsDNA	Enveloped, complex	Lumpy skin disease, Sheeppox, Fowlpox
Asfarviridae	dsDNA	Enveloped, icosahedral	African swine fever
Parvoviridae	ssDNA	Non-enveloped, icosahedral	Canine parvovirus, Feline panleukopenia
Picornaviridae	ssRNA+	Non-enveloped, icosahedral	Foot-and-mouth disease, Avian encephalomyelitis
Rhabdoviridae	ssRNA-	Enveloped, bullet-shaped	Rabies virus, Vesicular stomatitis
Paramyxoviridae	ssRNA-	Enveloped, pleomorphic	Rinderpest, Newcastle disease, Canine distemper
Orthomyxoviridae	ssRNA-	Enveloped, spherical	Influenza viruses (avian, equine, swine)
Retroviridae	ssRNA+	Enveloped, spherical	Feline leukemia virus, Equine infectious anemia
Reoviridae	dsRNA	Non-enveloped, icosahedral	Bluetongue virus, African horse sickness

Table 14. Continued.

Virus family	Nucleic acid	Structure	Example viruses/Diseases
Coronaviridae	ssRNA+	Enveloped, pleomorphic	Feline infectious peritonitis, Transmissible gastroenteritis
Papillomaviridae	dsDNA	Non-enveloped, icosahedral	Bovine papillomatosis, Equine papillomatosis

3.4. Transmission of animal viruses

Animal viruses are transmitted through various routes:

- **Direct contact**: Animal-to-animal contact or contact with infected tissues, secretions, or excretions.
- **Indirect contact**: Fomites (contaminated objects), or environmental contamination.
- **Aerosol/Respiratory**: Inhalation of virus-containing droplets. Examples include influenza viruses and Paramyxoviruses.
- **Vector-borne**: Arthropod vectors (mosquitoes, ticks, midges). Examples include the Bluetongue virus (Culicoides), African horse sickness (Culicoides).
- **Vertical Transmission**: Transplacental (in utero), via eggs in avian species. Examples include some parvoviruses, herpesviruses.
- **Alimentary/Fecal-oral**: Ingestion of contaminated food or water. Examples include Rotaviruses, some enteroviruses.

3.5. Viral pathogenesis and disease

The pathogenesis of viral infections involves:

a) **Entry and Primary Replication**: Initial infection at portal of entry.
b) **Spread**: Local or systemic dissemination.
c) **Cell/Tissue damage**: Through direct cytopathic effects or immune-mediated damage.
d) **Host response**: Innate and adaptive immune responses.
e) **Viral Clearance or Persistence**: Resolution or establishment of chronic/latent infection.

Disease manifestations vary widely:

- ✓ Respiratory diseases (e.g., Influenza).
- ✓ Enteric diseases (e.g., Rotavirus infections).
- ✓ Neurological diseases (e.g., Rabies).
- ✓ Vesicular diseases (e.g., Foot-and-mouth disease).
- ✓ Hemorrhagic diseases (e.g., African swine fever).
- ✓ Immunosuppressive diseases (e.g., Feline immunodeficiency virus).
- ✓ Oncogenic diseases (e.g., Avian leukosis).

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