Contents

Chapter 1

Ancient and Classic Biotechnology

1.1 Introduction

The term **biotechnology** was coined by Karl Ereky, known as the "father of biotechnology", in 1919. Biotechnology is defined as the use of biological organisms or their components as technologies in medicine, agriculture, and industry.

Historically, biotechnology has undergone three stages:

- **Ancient biotechnology:** Uses natural biotechnological functions for basic applications such as food and shelter;
- **Classic biotechnology:** Uses microorganisms and viruses in medical and industrial applications such as fermentation and vaccinations;
- **Modern biotechnology:** Uses advanced techniques such as genome sequencing for molecular-level applications.

1.2 Ancient Biotechnology

The earliest biotechnological application, occurring tens of thousands of years ago, was **domestication**, which increased animal supply and made animals easier to capture. Early domesticated animals include cattle, goats and sheep.

Domestication was followed by **farming** and related food storage methods.

Fermentation for products such as bread and cheese began within the past ten thousand years.

By the turn of the Common Era, biotechnological applications expanded to include primitive antibiotics, insecticides, and farming techniques such as crop rotation. Early theories of trait inheritance began to emerge.

Spontaneous generation, the idea that organisms arise from non-living matter, was the prevalent ideology prior to classic biotechnology.

1.3 Classic Biotechnology

1.3.1 Classic Biotechnology Events

1590: Zacharias Janssen and his son Hans invented the **microscope**;

1663: Robert Hooke recorded the first description of **cells**, found in plants;

1677: **Antonie van Leeuwenhoek** discovered living **microorganisms** - bacteria and protozoa;

1798: **Edward Jenner**, the "father of immunology", developed the first **vaccine**, for smallpox, after noticing that individuals exposed to cowpox were immune to smallpox. Jenner's work is said to have "saved more lives than any other man";

1824: Henri Dutrochet established that all living tissues are composed of cells;

1838: Gerardus Johannes Mulder discovered **protein**;

1859: Charles Darwin proposed **natural selection** to explain speciation and extinction;

1862: **Louis Pasteur** discovered that microorganisms cause **fermentation**;

1863: Gregor Mendel, the "father of modern genetics", discovered the laws of trait inheritance by experimenting on pea plants, and established the basic rules of **heredity**, referred to as "Mendelian inheritance";

1869: Friedrich Miescher discovered **DNA**;

1877: Robert Koch developed methods for bacteria **staining**;

1878: Walther Flemming discovered chromatin and **chromosomes**.

1.3.2 Pasteur and Fermentation

Louis Pasteur noted that the sugars in spoiled fermentation samples were converted to lactic acid instead of alcohol. Upon inspection, he discovered yeast cells in healthy fermentation samples, and bacteria in spoiled fermentation samples. This led him to conclude that fermentation is caused by microorganisms rather than being an innate chemical process, and that the spoiled samples were caused by bacterial growth.

Pasteur experimented further with boiled sugar water in a swan-necked flask. The boiling killed the microorganisms, and the swan-neck flask prevented microorganisms from entering. The sugar water did not ferment, demonstrating that fermentation is caused by microorganisms, and also disproves spontaneous generation.

Based on these observations, Louis developed **pasteurization**, which heats liquids to a temperature sufficient to kill microorganisms but not to significantly alter the food.

Pasteur also established the basic laws of glucose metabolism, namely that it can occur in both the presence and absence of oxygen, though with different processes. In **aerobic respiration**, the glucose is fully oxidized, and is converted to CO_2, H_2O, and energy; in **anaerobic respiration**, the glucose is not fully oxidized, and a fermentation byproduct, such as ethanol or lactic acid, is released.

Based on Pasteur's work, microorganisms were utilized to produce further products, such as glycerol, acetone, and citric acid.

In 1928, Alexander Fleming discovered **penicillin**, a byproduct of mold metabolism. In the 1940s, penicillin became the first mass-produced microbial product and antibiotic.

1.3.3 DNA as the Transforming Principle

In 1928, Frederick Griffith discovered the existence of a **"transforming principle"**. Griffith experimented with mice and two strains of bacteria - a virulent and lethal S-strain, and a non-virulent non-lethal R-strain. Injecting live S-strain into the mice caused them to die; injecting dead S-strain or live R-strain did not. However, when dead S-strain was mixed with live R-strain and injected, the mice died, and live S-strain was found in the dead mice. Griffith concluded that a "transforming principle" in the dead S-strain transformed the live R-strain into S-strain.

In 1941, Beadle and Tatum proposed the **"one gene, one enzyme"** hypothesis. They discovered through experiments on mutated molds that specific mutations caused the molds to lose the ability to synthesize specific enzymes, leading them to conclude that specific genes are responsible for producing specific enzymes, and when the genes are damaged, the corresponding enzymes cannot be produced.

In 1944, Avery, MacLeod, and McCarty expanded on Griffith's work and demonstrated that the transforming principle is DNA. They selectively removed chemical components of S-strain bacteria, such as proteins, lipids, RNA, and DNA, and discovered that transformation only occurred in the presence of DNA.

In 1952, **Hershey and Chase** definitively demonstrated that the transforming principle is DNA. They infected bacteria with bacteriophages with radioactively labeled DNA and protein, and discovered that only DNA from the infector strains was present in the infected strains, and not protein, demonstrating that DNA is what causes transformation.

1.3.4 Structure of DNA

In 1945, Erwin Chargaff discovered **"Chargaff's rule"**, which states that DNA contains an equal amount of adenine (A) and thymine (T), and an equal amount of guanine (G) and cytosine (C).

In 1953, Rosalind Franklin and Maurice Wilkins captured X-ray images of DNA.

In 1953, James Watson and Francis Crick used Chargaff's and Franklin's data to construct the DNA **double-helix** model, featuring two antiparallel sugar-phosphate strands forming the outer helix, and inner complementary base pair rungs, held together by hydrogen bonds.

1.3.5 Semiconservative Replication

In 1958, Matthew Meselson and Franklin Stahl demonstrated the **semiconservative** nature of DNA replication, in which each new double-strand contains one original and one new strand, by experimenting with tagged strands of DNA and observing that new double-strands contained one tagged and one untagged strand.

Chapter 2

Modern Biotechnology

2.1 Common Terms in Modern Biotechnology

FISH (Fluorescence in Situ Hybridization): Method of identifying genetic material through fluorescence labeling;

Pharmacogenetics: Study of how an individual's genetic makeup influences their response to medications;

CRISPR: Gene editing technology;

Hybridoma: Hybrid cell created by joining two different cell types;

Recombinant DNA: DNA formed from joining DNA fragments from different sources;

GMO: Genetically modified organism;

Transgenic organism: Organism genetically engineered to carry genes from a different species;

Animal bioreactor: Transgenic organism used to produce biomaterials.

2.2 Timeline of Modern Biotechnology

Individual genes that code for specific proteins were first identified in the 1970s, followed by early **genetic engineering**;

Genetic modification of food began in the 1990s;

By the late 1990s, **stem cell** experimentation had begun, and the first **cloned** mammal, "Dolly the Sheep", was created;

The **Human Genome Project** was mostly completed in the early 2000s, and fully completed around 2020;

In the 2020s, **artificial intelligence** is rapidly advancing biotechnology, including applications such as protein structure prediction.

2.3 Medical Applications of Modern Biotechnology

Gene therapy is the introduction, modification, or removal of genetic material for therapeutic purposes. **Vectors**, typically virus-derived, are used to deliver the genetic material to the affected cell. There are two categories of gene therapy:

- **In vivo** gene therapy takes place while the affected cells are still in the patient's body;

- **Ex vivo** gene therapy is performed on cells that have been removed from the patient's body, and are subsequently returned.

Stem cells are undifferentiated cells, and are used for tissue generation and research. There are several types of stem cells:

- **Embryonic stem cells** are derived from embryos and are **pluripotent** - they can differentiate into nearly any cell type;
- **Adult / tissue-specific stem cells** are found in specific tissues and are **multipotent** - they can only differentiate into a limited range of cell types;
- **Induced pluripotent stem cells** are adult stem cells reprogrammed to become pluripotent.

Transplantation is the transfer of tissue from one part of a patient's body to another, or between patients. **Xenotransplantation** is cross-species transplantation.

Artificial blood has life-saving potential but faces significant challenges. There are several potential pathways for creating artificial blood:

- **Hemoglobin-based** artificial blood must address the high toxicity of hemoglobin;
- **Perfluorocarbon-based** artificial blood is made from synthetic compounds but has limited effectiveness and potential side effects;
- **Stem cell-based** artificial blood may be a promising method and is being researched.

2.4 Genetic Testing

Gene testing examines an individual's DNA sequence, generally to determine if the individual carries a gene associated with illnesses such as Parkinson's disease, celiac disease, or blood clotting disorders. This can help prevent, diagnose, or treat diseases.

A **karyotype** is an image of an individual's entire set of chromosomes. **Chromosomal testing** examines an individual's karyotype for abnormalities in chromosomal number, size, or structure. Chromosomal abnormalities can cause systemic health and developmental disorders.

Antibody testing detects the presence of antigens. There are two types of antibodies:

- **Monoclonal antibodies** are produced from a single B-cell hybridoma. All antibodies produced from the hybridoma are identical and bind to the same antigen epitope;
- **Polyclonal antibodies** are harvested from animals. The antibodies are not identical, as they are produced by different B-cells, and do not bind to the same antigen epitope.

Biochemical testing examines the presence of specific proteins by analyzing substrate catalysis, and is used for:

- Genetic testing, such as screening newborns for genetic disorders that affect protein production;
- Identifying organisms or cells based on their protein profile.

Prenatal tests are performed on fetuses. Methods of prenatal testing include:

- **Amniocentesis**: Tests a portion of amniotic fluid;
- **Chorionic villus sampling (CVS):** Tests a portion of the placenta's chorionic villi.

2.5 Further Applications of Modern Biotechnology

Further modern biotechnological applications extend to:

- **Pharmaceutical** manufacturing of drugs such as insulin;

- **Drug delivery methods** such as liposomes;
- **Agricultural** applications, such as artificial insemination, animal vaccines, and single-cell protein for animal feed;
- **Genetically modified crops** designed to increase yield and enhance resistance to diseases, pesticides, and extreme temperatures;
- **Environmental** applications, such as microbes used for waste management, enzymes used for cleaning, and transgenic malaria-resistant mosquitoes;
- **Food** applications, such as alternative sweeteners and fat substitutes;
- **Forensics**, such as DNA profiling and genetically tracing disease origins;
- **Research**, such as creating transgenic animals for pharmaceutical production and knockout lab mice for research purposes.

Genetically modified agricultural products are classified into three categories:

- **First generation** products focus on increased crop resistance and are primarily used in products such as animal feed and byproducts such as oil;
- **Second generation** products focus on quality improvement such as enhanced appearance or nutrition and are mostly used for human food products;
- **Third generation** products are an emerging field and utilize modified plants to produce non-food products such as pharmaceuticals.

Bioinformatics employs large-scale data sets, computational tools, and artificial intelligence to study genetic information in order to find the genetic causes of diseases and to personalize treatments.

The **Human Genome Project** was a global initiative to sequence the entire human genome. Human DNA contains roughly 3 billion base pairs and 20,000-25,000 genes.

Chapter 3

Mitosis and Meiosis

3.1 Overview of Mitosis and Meiosis

Human DNA is organized into 23 chromosomes.

The standard **diploid** cell contains two sets of chromosomes, one from each parent, with a total of 46 chromosomes. Some cells - such as fully differentiated blood cells - are anucleate and do not contain DNA.

Gametes / sex cells - female's ova and male's sperm - are **haploid cells** and only contain a single set of DNA. A gamete from each parent combines to form a child, giving the child two sets of DNA, one from each parent.

Germ cells are diploid cells that produce gametes. All other diploid cells are **somatic cells**.

Mitosis is the process in which a cell replicates its DNA and splits into two **daughter cells** that are identical to each other and itself.

Purposes of mitosis include:

- Growth
- Repair
- Maintenance
- Single-celled organism asexual reproduction

Although asexual reproduction is simpler than sexual, the child organism is identical to the parents and lacks genetic variety. Sexual reproduction combines the DNA of two parents, increasing genetic variety.

Most somatic cells undergo mitosis. **Terminally differentiated cells** - including nerve and muscle cells - do not undergo mitosis and die at the end of their life cycle.

Meiosis is the process in which diploid germ cells divide to form four haploid gametes.

3.2 Interphase

The majority of the cell's life cycle is occupied by **interphase**, in which the cell performs most of its tasks, and occurs prior to mitosis and meiosis.

The cell replicates its DNA during interphase.

Interphase is divided into G1, S, and G2. In G1, the cell performs its normal functions, and prepares for replication, which occurs in the S phase. In G2, the cell prepares for mitosis.

3.3 Stages of Mitosis

The four stages of mitosis are:

- **Prophase:** During interphase, DNA is uncoiled as loose chromatin. By prophase, the chromatin condenses into distinct chromatid structures. Each pair of identical sister chromatids is joined at the centromere, forming the characteristic single X-shaped chromosome structure;
- **Metaphase:** During metaphase, the chromosomes line up in the middle of the cell;
- **Anaphase:** During anaphase, the sister chromatids are pulled apart to opposite ends of the cell; 46 chromatids to each end;
- **Telophase:** During telophase, the chromatids are on opposite ends of the cell, and new nuclei form around them.

After mitosis is complete, **cytokinesis** takes place, during which the cell splits into two new cells, each with a single nucleus containing a complete set of 46 chromosomes.

3.4 Stages of Meiosis

The interphase preceding meiosis in germ cells resembles the interphase of somatic cells, involving DNA replication and preparation for division.

Meiosis consists of two stages, **Meiosis I** and **Meiosis II**, each with four phases, totaling eight phases:

- **Prophase I;**
- **Metaphase I;**
- **Anaphase I;**
- **Telophase I;**
- **Prophase II;**
- **Metaphase II;**
- **Anaphase II;**
- **Telophase II**.

During prophase I, **chiasmata** occurs, in which homologous chromosomes (one from each parent) cross over and exchange genetic material and create recombinant chromatids. This increases genetic diversity.

During anaphase I, the homologous chromosomes are separated to two daughter cells, though the sister chromatids remain intact. Each daughter cell is haploid with 23 chromosomes; each chromosome has two identical chromatids.

During anaphase II, the sister chromatids of each daughter cell are split, forming four total daughter cells. Each daughter cell has 23 chromosomes; each chromosome has one chromatid.

3.5 Diseases Resulting from Errors in Meiosis

In **normal disjunction**, chromosomes divide properly, resulting in four daughter cells, each with one chromosome.

In **nondisjunction**, chromosomes fail to separate properly, resulting in one daughter cell with two chromosomes and one with none.

Nondisjunction can occur during meiosis I – **first division nondisjunction** – or meiosis II – **second division nondisjunction**.

An offspring from the daughter cell with two chromosomes is **trisomic**, with three total chromosomes; an offspring from the daughter cell with no chromosomes is **monosomic**, with one total chromosome.

Nondisjunction disorders include:

- **Down syndrome:** Trisomy 21; caused by an extra 21st chromosome. Traits include short stature, distinct facial features, and limited mental capability;
- **Klinefelter's syndrome:** XXY sex chromosome. Presents as an infertile male with feminine traits, low intelligence, and above-average height;
- **Turner's syndrome:** XO sex chromosome. Presents as an infertile female with average intelligence and below-average height.

3.6 Genetic Diseases Resulting from Errors in DNA Replication

Cancer is a disease of mitosis in which cancerous cells divide uncontrollably, bypassing mitotic checkpoints.

Errors in mitosis may also contribute to neurodegenerative diseases, such as Alzheimer's disease.

Genetic diseases generally originate from an error during pre-meiosis S-phase, causing defective DNA in the gamete that results in a child with a defective genome. These errors include:

- **Deletion:** A section of the chromosome is deleted;
- **Duplication:** A section of the chromosome is duplicated;
- **Inversion:** A section of the chromosome is reversed;
- **Translocation:** A section of the chromosome is translocated to another chromosome.

If a genetic disease is **recessive**, it only manifests if both copies of DNA are defective; if it is **dominant**, it manifests if even one copy of DNA is defective.

Traits linked to the first 22 chromosomes are **autosomal traits**. Traits linked to the 23rd chromosome are **sex-linked traits**. Males, having only one X chromosome, are more likely to express X-linked recessive diseases because they lack a second X chromosome to compensate for a defective gene.

Common genetic diseases include:

- **Cystic fibrosis:** Causes the production of thick, sticky mucus, affecting the lungs and digestive systems;
- **Sickle cell anemia:** Abnormal hemoglobin causes red blood cells to assume a sickle shape, potentially blocking capillaries;
- **Hemophilia:** Impairs blood clotting due to defective clotting factors;
- **Huntington's disease:** Leads to the production of a toxic protein that degenerates the nervous system;
- **PKU (phenylketonuria):** Causes a deficiency of phenylalanine hydroxylase which leads to toxic levels of phenylalanine and may cause mental retardation;
- **Color blindness;**
- **Muscular dystrophy:** Causes progressive muscle weakness and degeneration.

3.7 Pedigree Analysis

Pedigree analysis involves constructing a family tree to chart the inheritance of a genetic trait, and can be used to determine if a trait is dominant or recessive and if it is autosomal linked or sex linked.

In a pedigree, males are represented by squares, females by circles, and individuals with the trait are shaded. A horizontal line between a male and female indicates mating, and vertical lines connect to their offspring.

If males and females are affected equally, the trait is likely autosomal. If it primarily affects males, the trait is likely sex-linked.

If members in every generation are affected, the trait is likely dominant. If generations are skipped, the trait is recessive.

3.8 Gene Therapy

Gene therapy is the insertion of therapeutic genetic material to treat or prevent disease, including:

- Replacing missing or mutated genes;
- Inactivating harmful genes;
- Regenerating tissue;
- Targeted cancer therapy;
- Genetic vaccination.

Gene therapy typically uses **vectors**, such as modified viruses, to deliver therapeutic genes into cells.

There are two types of gene therapy:

- **Somatic gene therapy:** Treatments are not inherited by the individual's offspring;
- **Germ line gene therapy:** Germ cells are treated, resulting in treated gametes that are passed to the individual's offspring.

Gene therapy is used to treat diseases such as cystic fibrosis, heart disease, and cancer.

Chapter 4

Immunology

4.1 The Immune System

Overview of the Immune System

The **immune system** protects the body from pathogens, harmful chemicals, and cancerous cells.

Antigens are molecules that trigger an immune response. An **epitope** is the part of an antigen that the immune system recognizes as foreign.

The immune system is composed of three lines of defense:

- The **first line of defense** includes physical barriers, such as the skin, and chemical barriers, such as mucus secretions;
- The **second line of defense**, the **innate immune system**, is non-specific to antigens and present at birth;
- The **third line of defense**, the **adaptive immune system**, is highly specific and adapts to encountered antigens;
- The second and third lines of defense are primarily composed of white blood cells.

Components of the Innate Immune System

Inflammation and **fever** are responses of the innate immune system:

- **Inflammation:** Chemicals such as histamine are released to recruit immune cells. Signs include pain, redness, and swelling;
- **Fever:** Body temperature rises, increasing blood flow and denaturing the proteins of pathogens.

Allergies are innate immune system responses to **allergens** - non-pathogenic antigens.

Phagocytes, a type of white blood cell, are a component of the innate immune system that initiate an immune response, ingest antigens, and present fragments of digested antigens to **helper T-cells**, triggering the adaptive immune system.

Components of the Adaptive Immune System

Helper T-cells activate cytotoxic T-cells and B-cells.

Activated **cytotoxic T-cells** directly attack pathogens.

Activated **B-cells** differentiate into **plasma cells** which produce **antibodies (immunoglobulins)**.

Some helper T-cells, cytotoxic T-cells, and B-cells differentiate into **memory** helper T, cytotoxic T, and B cells.

The **primary response** is the immune system's initial encounter with a specific antigen, during which memory cells are created. The **secondary response** is subsequent encounters with the antigen, and is quicker and stronger than the primary response due to the memory cells.

Apoptosis

Apoptosis is the process in which a cell self-destructs.

- Some components of the innate and adaptive immune system induce antigen apoptosis.
- Many infected cells undergo apoptosis.

4.2 Antibodies

Antibodies are proteins of the adaptive immune system that bind to specific epitopes of antigens through their antigen-binding regions.

Composition: Y-shaped, consisting of two **heavy chains** and two **light chains** stabilized by **disulfide bridges**. There are **variable regions** at the end of the chains specific for the targeted epitope.

Classes of antibodies include:

- **IgG:** Most common type of antibody and the one to cross the placenta;
- **IgA:** Found in body secretions;
- **IgD:** Helps activate B-cells;
- **IgE:** Responds to parasites;
- **IgM:** Activates complement.

Antibodies circulate the bloodstream and bind to antigens they encounter, forming an **antibody-antigen complex** that aid the immune response through the following mechanisms:

- **Neutralization:** Directly neutralizing the antigen;
- **Precipitation** and **agglutination:** Antibodies link multiple antigens into large complexes, precipitating soluble antigens or agglutinating non-soluble ones. These complexes are easier for immune cells to target;
- **Complement fixation:** Antibody-antigen complexes activate the complement system, leading to the lysis of antigens.

4.3 Immunization

Immunization is the process of rendering an individual immune to an antigen.

Types of immunity include:

- **Active immunity**, in which the individual gains memory B and T cells;
- **Passive immunity**, in which the individual gains antibodies.

Vaccines are a method of gaining active immunity through exposure to an inactivated (killed) or attenuated (weakened) pathogen, and may require **booster** doses to sustain active immunity and ensure continued antibody production.

4.4 Autoimmune Diseases

Autoimmune diseases are disorders in which the body mounts an immune attack against itself. Autoimmune diseases can have diverse triggers, and their precise causes are not well understood.

Common autoimmune diseases include:

- **Multiple sclerosis (MS):** Affects nerves' myelin sheath;
- **Type-1 diabetes:** Affects cells that produce insulin;
- **Celiac disease:** Reacts to gluten;
- **Lupus:** Affects multiple tissues and causes widespread inflammation;
- **Psoriasis:** Causes skin cells to rapidly multiply, forming thick, scaly skin patches;
- **Crohn's disease:** Affects the digestive tract;
- **Rheumatoid arthritis:** Affects joints.

4.5 Diagnostics

Serology is the study of antigen-antibody interactions in a solution.

Precipitation and **agglutination** tests detect antibodies or antigens by testing for an antibody-antigen precipitation or agglutination reaction.

- Precipitation tests require a precise antigen-to-antibody ratio within the **zone of equivalence**.
- **Hemagglutination** is an agglutination test performed with red blood cells to determine blood type.

Virus neutralization assays measure the presence of neutralizing antibodies by assessing a sample's ability to prevent viral infection of cells.

ELISA (enzyme-linked immunosorbent assay) detects a target molecule by introducing a primary antibody binded to an enzyme that catalyzes a color change in the solution.

- **Horseradish peroxidase (HRP)** is commonly utilized as the ELISA enzyme.

Types of ELISA include:

- **Sandwich ELISA:** A fixed antibody binds to the target molecule, which binds to an enzyme-linked antibody;
- **Direct ELISA:** The target molecule binds directly to an enzyme-linked antibody;
- **Competitive ELISA:** The sample competes with enzyme-linked molecules for binding; lower enzyme activity indicates higher target concentration.

In **Western blot assays** separate proteins by size and introduce a labeled antibody, indicating the size of the target molecule.

Immunofluorescence assays detect a target molecule by introducing fluorophore-labeled antibodies.

Immunochromatographic assays, commonly used in over-the-counter kits such as those for COVID-19 and pregnancy, detect target molecules using visible lines formed by pigment-labeled antibodies. The sample flows through a test strip via capillary action and encounters free pigment-labeled primary antibodies and a line of immobilized primary antibodies. If the target molecule is present, it is sandwiched between the two antibodies, causing pigment accumulation to form a visible line. A control line is often used, made of immobilized secondary antibodies that directly bind to the primary pigment-labeled antibodies.

Chapter 5

Virology

5.1 Viruses

Virus components include:

- **Genetic material:** DNA or RNA;
- **Capsid:** Protein coat;
- **Lipid envelope:** Some viruses have a lipid envelope, derived from their host cells' membranes.

Viruses infect living cells, and their genome (DNA or RNA) hijacks the host cell's machinery for viral replication, often harming the host.

Viruses do not belong to any of the kingdoms of life and lack several characteristics of life; there is debate as to whether they are "alive."

Retroviruses are a class of viruses that use **reverse transcriptase**, an enzyme, to transcribe their RNA genome into DNA, integrating it into the host's genome.

- The reverse transcriptase gene is used to manufacture reverse transcriptase for laboratory use in applications such as qRT-PCR (quantitative reverse transcription polymerase chain reaction).

Viral **transmission** refers to the mechanisms by which a virus spreads between hosts.

Needlestick injuries occur when contaminated needles transmit viruses, often due to improper practices like recapping in healthcare settings.

Viral **pathogenesis** describes how a virus induces disease. **Acute** infections develop rapidly and resolve quickly; **chronic** infections persist over an extended period.

The **viral antigen** is the component of the virus recognized by the immune system and antibodies. **Seroconversion** refers to the production of detectable antibodies in the blood following exposure to an antigen, either through infection or vaccination. The **seroconversion rate** is the percentage of people who develop antibodies in response to a given antigen. **Antibody titer** measures the concentration of antibodies in serum.

5.2 Varicella-Zoster Virus

The **varicella-zoster virus (VZV)** causes varicella, commonly known as chickenpox, primarily in children. Following acute varicella infection, VZV can establish latency in sensory ganglia and may later reactivate as zoster (shingles), particularly in older or immunocompromised individuals.

Primary mode of transmission: Airborne respiratory droplets.

Viral features: Enveloped, double-stranded DNA virus.

Diagnosis: A fluorescent antibody test is used to identify VZV in cell culture.

Treatment / prevention:

- The varicella vaccine is a live, attenuated virus;
- Immunocompromised individuals may receive antiviral therapy and varicella-zoster immune globulin (VZIG) for passive immunity;
- The shingles vaccine is recommended for individuals over 50 with a history of varicella.

5.3 Hepatitis B

Hepatitis viruses primarily target the liver.

Common types of hepatitis include hepatitis A, B, and C. All types of hepatitis can cause acute inflammation of the liver, which may lead to symptoms such as jaundice. Types B and C can also lead to chronic hepatitis.

Viral features: Enveloped virus with partially double-stranded DNA and reverse transcriptase.

Mode of transmission: Bodily fluids, including:

- Blood;
- Semen;
- Cervicovaginal secretions;
- Rectal secretions;
- Breast milk.

Potential diseases caused by HBV:

- Hepatocellular carcinoma;
- Hepatocellular injury resulting from a cytotoxic T-cell-mediated immune response to infected liver cells.

HBV expresses three key antigens:

- **Surface (HBsAg):** Forms rods and spheres in serum that indicate active infection and is used in initial testing. The presence of HBsAg for over six months indicates chronic hepatitis. HBsAb (anti-HB) indicates HBV immunity;
- **Core (HBcAg):** HBcAg is part of the HBV nucleocapsid, is expressed during HBV replication within liver cells, and is not detectable in the patient's serum. However, HBcAb is detectable and indicates immunity. Though HBsAb can result from artificial immunity (vaccination), HBcAb can only result from natural immunity;
- **E (HBeAg):** HBeAg is a secreted antigen that reflects active viral replication in the liver and indicates high infectivity.

HBV prevention / treatment:

- HBsAg Vaccination;
- Passive immunity through HBsAb;
- Public education on minimizing HBV transmission.

5.4 RSV

RSV (respiratory syncytial virus) is a common respiratory virus that causes neighboring respiratory cells to fuse and form syncytia - multinucleate clumps.

Symptoms are generally mild but can be serious, especially in infants and the elderly.

Primary mode of transmission: Airborne respiratory droplets.

5.5 Coronavirus

Coronavirus is a common virus and is the second-most frequent cause of the common cold.

Coronaviruses primarily cause upper respiratory tract infections. A unique symptom of the COVID-19 strain of coronavirus is the loss of smell and taste.

Coronavirus is **zoonotic** - can be transmitted between species, though it typically infects specific host species.

Primary mode of transmission: Airborne respiratory droplets.

Coronavirus features: Single-stranded RNA (the largest viral RNA genome), envelope.

Coronavirus contains four structural proteins:

- **Spike (S):** Glycoprotein spikes protruding from the envelope, giving a crown-like (corona) appearance. These spike proteins recognize and bind to **ACE2** receptors on host cells;
- **Membrane (M);**
- **Envelope (E);**
- **Nucleocapsid (N).**

These proteins, particularly the spike protein, mutate frequently, complicating diagnostics, therapy, and vaccine development.

5.6 HIV

Human Immunodeficiency Virus (HIV) is a virus that can lead to **Acquired Immunodeficiency Syndrome (AIDS)**, which targets CD4 T-cells.

The seroconversion rate for HIV is 0.3%.

Viral features: Single-stranded RNA genome with reverse transcriptase.

Transmission: Body fluids.

Treatment: Antiretroviral therapy (ART) is a daily combination of drugs that suppresses HIV, reducing transmission and progression to AIDS, though it does not cure the virus. ART drugs include:

- Reverse transcriptase inhibitors;
- Integrase inhibitors;
- Protease inhibitors.

Chapter 6

Fermentation

6.1 Fermentation Overview

Fermentation is a biochemical process in which microorganisms break down glucose to produce ATP in the absence of oxygen. The glucose undergoes glycolysis and is converted to two pyruvic acid molecules, producing ATP. This process converts two NAD^+ molecules into NADH. To revert the NADH to NAD^+ so that further fermentation can occur, the pyruvic acids are converted into byproducts, such as ethanol, lactic acid, or carbon dioxide, depending on the specific microorganism.

The applied definition of fermentation refers to **industrial microbiology**, in which microorganisms are used to produce products of economic value. This is generally done on a large scale with standardized procedures.

Fermentation products fall into five categories:

- **Microbial cells / biomass:** The microorganisms themselves are the product, such as single-cell protein, baker's yeast, and *E. coli*;
- **Microbial enzymes;**
- **Metabolites:** Products synthesized by the microorganisms. There are two types of metabolites:
  - **Primary metabolites** are produced as part of normal growth and development, and are produced during the log phase. Examples include ethanol and citric acid;
  - **Secondary metabolites** are not produced as a part of normal growth and development, and are produced during the stationary phase. Includes antibiotics, which are produced to combat potentially competing species rather than innate growth and development.
- **Recombinant products** are produced by microorganisms that have been infused with genetic material from another species, such as insulin;
- **Biotransformation:** Substrates are converted into modified products, such as steroid transformations.

Examples of common fermentation products and uses include:

- Ethanol for beer, wine, and biofuel;
- Acetic acid for vinegar;
- Lactic acid for sour products such as cheese, rye bread, and sauerkraut;
- Acetone, butanol, and glycerol for pharmaceutical and industrial uses;
- Citric acid for flavoring;

- Methane for fuel;
- Sorbose for vitamin C.

6.2 Materials and Methods

Key components of fermentation include:

- **Media / substrate:** The feed solution containing essential nutrients for microbial growth, such as water, carbon, nitrogen, and minerals. Examples include cane molasses, beet molasses, and cereal grains;
- **Starting material:** The material the microbes are to metabolize. Often serves as the media as well;
- **Inoculum / starter culture:** The initial microbial colony introduced to the fermentation process.

Fermentation is typically performed in a **bioreactor / fermenter**. Components of industrial fermenters include:

- Computer control panel;
- A **sparger**, which injects high-pressure air for **aeration**;
- A motor and impeller blades to mix the product;
- Temperature regulation system through exterior cooling jackets or internal cooling coils;
- pH controller with an acid-base reservoir and pump;
- Oxygen-level controls;
- Exhaust to remove waste gas.

6.3 Steps of Fermentation

Upstream processes are preparatory steps performed prior to fermentation, including:

- Obtaining, storing, and sterilizing raw material;
- Inoculum development: Often performed in a dedicated **seed fermenter**;
- Sterilizing the fermenter and pipework, typically achieved by flushing with superheated steam.

Fermentation operational modes include:

- **Batch process:** Media and inoculum are added, left to ferment, and drained once the product forms. Includes penicillin production;
- **Continuous process:** Media is continuously added to and withdrawn from the fermenter. Includes ethanol and beer production.

Microbial growth is divided into four phases:

- **Lag phase:** Initial phase with minimal microbial growth as they acclimate to the environment;
- **Log phase:** Exponential growth;
- **Stationary phase:** Population reaches equilibrium;
- **Death phase:** Population dies as resources deplete.

Downstream processes are finalizing steps performed after fermentation, including:

- **Cell disruption:** Harvesting intracellular products;
- Isolation and purification;

- Post-processing steps, such as crystallization and concentration;
- Storage and packaging.

Chapter 7

Regulation

7.1 Federal Regulatory Agencies

Federal agencies play critical roles in regulating food, drug safety, public health, and related activities.

Food and Drug Safety

- **FDA (Food and Drug Administration):** Regulates pharmaceutical drugs;
- **USDA (United States Department of Agriculture):** Regulates agricultural products.

Public Health and Healthcare

- **DHHS (Department of Health and Human Services):** Oversees public health and essential human services;
- **HCFA (Health Care Financing Administration):** Manages Medicare and Medicaid programs;
- **CDC (Centers for Disease Control and Prevention):** Focuses on disease prevention and control;
- **USPHS (United States Public Health Service):** Promotes public health and safe practices.

Workplace and Environmental Safety

- **EPA (Environmental Protection Agency):** Regulates environmental hazards;
- **OSHA (Occupational Safety and Health Administration):** Oversees workplace safety and laboratory practices.

Hazardous and Radioactive Materials

- **DOT (Department of Transportation):** Oversees the transport of potentially hazardous materials;
- **NRC (Nuclear Regulatory Commission):** Regulates the use of radioactive materials in energy and medical applications.

7.2 Lab Practices

Personal Safety Guidelines

Prohibited Activities:

- No smoking;
- No makeup application;
- No mouth pipetting.

Protective Gear:

- Wear protective gear, such as goggles, gloves, lab coats, and face shields;
- Remove all protective gear and wash hands and forearms after finishing work.

Familiarity with Safety Equipment:

- Know the location of alarms, exits, fire blankets, eye washers, and emergency showers.

Chemical Safety

Handling Chemicals:

- Use fume hoods when dealing with hazardous chemicals;
- Clean up spills immediately.

Chemical Spill Procedure (SWIMS):

- **S**top;
- **W**arn others;
- **I**solate the area;
- **M**inimize exposure;
- **S**urvey the area.

Laboratory Barriers

Primary barriers are internal laboratory barriers designed to protect laboratory workers, such as physical barriers and protective gear.

Secondary barriers are designed to protect the external environment, such as ventilation systems and airlocks.

Material Storage

- Space incompatible items, such as corrosives, flammables, and toxins;
- Follow container material and size regulations;
- Strap compressed gases;
- Store biohazards in designated biohazard cabinets (**BHC**).

Hazard Identification

The **NFPA 704 Standard** is a color-coded diamond system with 0-4 severity ratings:

- **Blue** indicates health hazard;
- **Red** indicates flammability;
- **Yellow** indicates reactivity;
- **White** indicates other hazards, such as water reactivity or oxidation.

Hazard-indicating pictograms include:

- Flammable;
- Harmful / irritant;
- Corrosive;
- Poisonous / toxic;
- Explosive;
- Biohazard;
- Oxidizer;
- Environmental hazard;
- Radioactive.

Labs must display a **biohazard level sign** indicating the **biohazard level**.

Laboratory Standards

Standards ensure safety, reliability, consistency, and reproducibility.

- **SOP** (standard operating procedures) are step-by-step instructions for laboratory tasks.
- **GLP** (good laboratory practices) are standards for study design, implementation, and documentation.

Additional standards include:

- **GDP:** Good documentation practices;
- **GMP:** Good manufacturing practices;
- **GCP:** Good clinical practices.

Universal precautions is a precautionary approach that treats all bodily fluids as potentially infected to prevent accidental transmission.

Documentation

It is essential to maintain a **lab notebook** and record all experiment details to a degree that enables precise replication.

7.3 OSHA

OSHA guidelines address workplace safety for conditions and materials that may pose physical or health hazards.

Types of Hazardous Materials

Types of hazardous materials and common examples include:

Physical hazards:

- **Flammable materials:** Ethanol.
- **Combustible materials:** Propylene glycol.
- **Compressed gases:** Oxygen.

Acute health hazards (have immediate effects):

- **Cryogens:** Liquid nitrogen.
- **Corrosives:** Hydrochloric acid.
- **Toxins:** Carbon monoxide.

Chronic health hazards (effective upon extended exposure):

- **Teratogens:** May cause developmental abnormalities (e.g., thalidomide).
- **Carcinogens:** May lead to cancer (e.g., asbestos).
- **Toxins:** Mercury.

Exposure Limits and Measurement

The **PEL** (permissible exposure limit) regulates the maximum a worker may be exposed to hazardous material. There are two categories of PELs:

- The **ceiling value** relates to acute hazards and is the maximum a worker may be exposed to the material at any given time;
- The **TWA** (time-weighted average) relates to chronic hazards and is the maximum average a worker may be exposed to on a regular basis.

LD50 (lethal dose 50%) is a toxicological measurement indicating the milligrams of substance per kilogram of body weight that is lethal for 50% of the population.

Protective Gear

OSHA requires the use of protective gear when working with hazardous substances, including:

- Eye protection;
- Face shields;
- Lab coats;
- Gloves;
- Fume hoods.

Material Safety Data Sheets (MSDS)

OSHA mandates that **MSDS**s be provided for all hazardous chemicals in the workplace. MSDS includes:

- Chemical and manufacturer identification;
- Composition;
- Physical and chemical properties;
- Hazard identification;
- Stability, reactivity, and toxicological information;
- Handling and storage requirements;
- Accidental release measures.

7.4 Bloodborne Pathogens Standards

OSHA's Bloodborne Pathogens Standards focus on bloodborne pathogens and include:

- Precautionary measures to prevent exposure;
- Infection control plans;
- Training and documentation.

7.5 CLIA

The **Clinical Laboratory Improvement Amendments (CLIA 1988)** were passed by Congress in 1988 to regulate *in vitro* testing facilities and personnel, ensuring testing reliability.

Testing facilities are classified into three categories, based on their test complexity and risk:

7.5.1 Waived Facilities

Waived facilities perform simple, low-risk tests, such as blood glucose, ovulation, and pregnancy tests.

Requirements:

- CLIA waiver certificate;
- No personnel requirements.

7.5.2 Moderate Risk Facilities

Moderate risk facilities perform tests with minimal preparation and procedural steps and limited operator intervention and results interpretation.

Requirements:

- CLIA certificate of accreditation;
- Proficiency testing;
- Quality control measures and SOPs;
- The following personnel:
  - Laboratory director;
  - Technical consultant;
  - Clinical consultant;
  - Testing personnel.

7.5.3 High Risk Facilities

High risk facilities perform tests with complex preparatory procedures and multiple steps, require operator intervention and interpretation of results, and pose patient risks if errors occur in results or procedures.

Requirements:

- All moderate risk facility requirements;
- General supervisor;
- Enhanced quality control protocols;
- Stringent record-keeping requirements.

7.5.4 Point-of-Care Tests (POCT)

POCT are rapid diagnostic tests performed at the patient care sites instead of in a laboratory. POCT benefits include portability, low cost, and speed of results; limitations include lower quality and lack of laboratory documentation and management systems.

7.6 Animal Research

Animal research has contributed to significant biomedical advancements, particularly in studying systems similar to humans, such as the immune system of mice and the cardiovascular system of dogs.

Mice and other rodents make up over 90% of animals used in research.

Before using animals, research must:

- Review comparable prior data;
- Conduct computer simulations (*in silico* methods);
- Obtain Institutional Animal Care and Use Committee (IACUC) approval;
- Complete extensive training.

The "three R's" of ethical animal research are:

- **Replace** animals wherever possible;
- **Reduce** the number of animals used;
- **Refine** tests to minimize animal stress.

Animal research is regulated by more agencies and laws than human research, including:

- Procurement;
- Transport;
- Housing;
- Treatment;
- Experimentation methods;
- Health maintenance;
- Euthanasia.

Euthanasia is the humane, rapid, and minimally distressing killing of an animal.