Principles of Biotechnology II

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Level 1
Second Semester
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Lec.1

Isolation of Microorganisms from the Environment and Their Nutritional Requirements

- **Introduction**

Microorganisms are the most diverse and numerous forms of life on Earth (Fig.1), found in almost every environment, from the depths of the ocean to the human body. These organisms play a crucial role in maintaining ecological balance, driving biogeochemical cycles, and contributing to the health of the planet. Understanding their nutritional requirements is essential for both environmental microbiology and biotechnology applications.

Microorganisms are isolated from environmental sources such as soil, water, air, and living organisms using different methods, depending on the type of microorganism and its habitat.

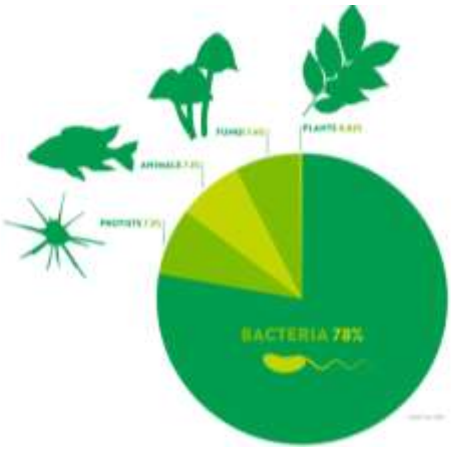

Figure 1: The ratio of microbiota on the earth

1. The Role of Microorganisms in the Environment

- **Decomposers**: Microorganisms, particularly bacteria and fungi, are key decomposers in ecosystems. They break down dead organic matter, releasing nutrients back into the environment and promoting the recycling of carbon, nitrogen, sulfur, and phosphorus.

- **Nitrogen Fixation**: Certain bacteria, such as *Rhizobium* species, form symbiotic relationships with plants and fix atmospheric nitrogen, converting it into a form usable by plants. This is crucial for soil fertility (Fig.2).

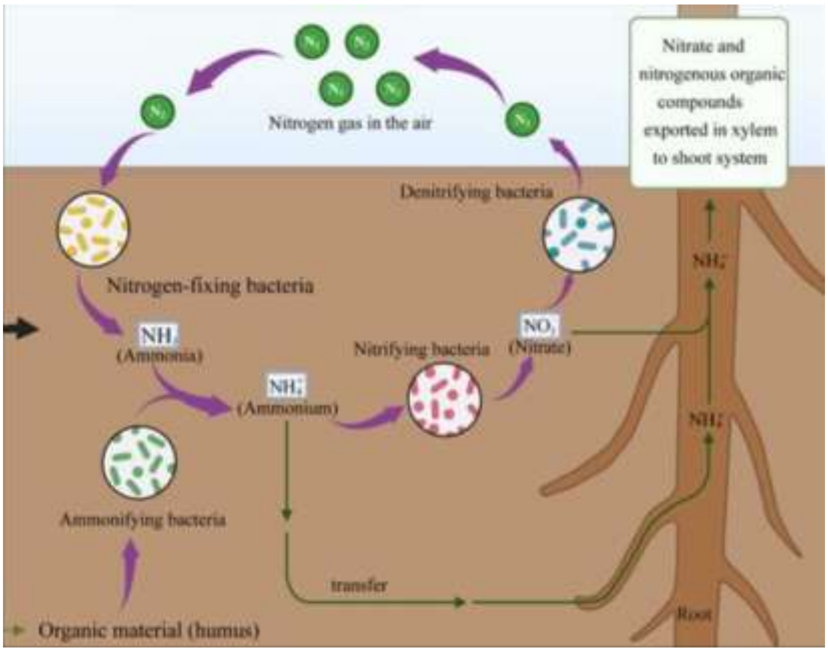

Figure 2: Microorganism participate in the nitrogen cycling processes in ecosystems.

- **Bioremediation**: Some microorganisms can degrade toxic pollutants in the environment, such as heavy metals and hydrocarbons. This process, known as bioremediation, is a sustainable and eco-friendly way to clean up contaminated environments (Fig.3).

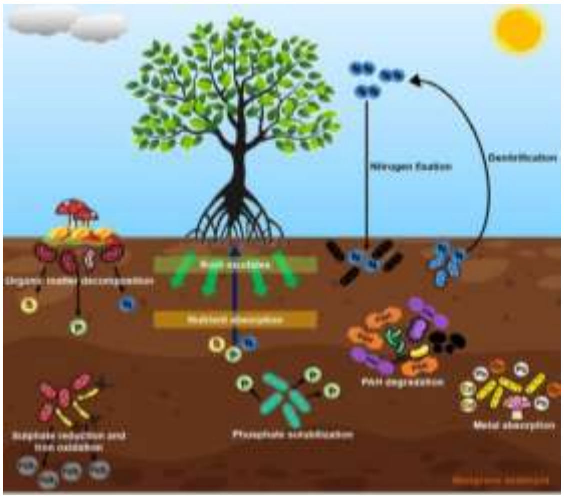

Figure 3: Microbial ecological interactions

- **Pathogenic Role**: While many microorganisms are beneficial, some can cause diseases in plants, animals, and humans. Understanding their behavior and environmental interactions is essential for controlling infections.

2. Nutritional Requirements of Microorganisms

Microorganisms require specific nutrients to grow and carry out their metabolic activities. These nutrients can be categorized into **macronutrients** and **micronutrients**.

Macronutrients

Macronutrients are required in large amounts to support the basic metabolic functions of microorganisms

- **Carbon (C)**: All living organisms require carbon for the synthesis of organic molecules, including proteins, lipids, carbohydrates, and nucleic acids. Microorganisms obtain carbon from organic sources (heterotrophs) or carbon dioxide (autotrophs).
    - **Heterotrophs**: These microorganisms rely on organic carbon sources like sugars, fats, and proteins.
    - **Autotrophs**: These organisms use inorganic carbon, primarily carbon dioxide, to synthesize organic molecules.
- **Nitrogen (N)**: Nitrogen is a key element in amino acids, proteins, and nucleic acids. Microorganisms obtain nitrogen from various sources, including atmospheric nitrogen (N_2) through nitrogen fixation, organic matter (proteins, peptides), and inorganic compounds (nitrates, ammonium).
- **Phosphorus (P)**: Phosphorus is essential for nucleic acids (DNA/RNA), phospholipids in cell membranes, and energy transfer molecules like ATP. Microorganisms obtain phosphorus from inorganic salts (e.g., phosphate) or organic molecules (e.g., nucleotides).
- **Sulfur (S)**: Sulfur is an important component of amino acids (like cysteine and methionine) and vitamins. Sulfur is available in the environment through the reduction of sulfate (SO_4^{2-}) or through organic sulfur compounds.
- **Potassium (K), Magnesium (Mg), Calcium (Ca)**: These elements act as cofactors for enzymes, contribute to maintaining osmotic balance, and help with cellular structure and signaling.

Table1 : Types of Microorganisms Based on Nutritional Needs

Type	Energy Source	Carbon Source	Electron Source	Example
Phototrophs	Light	CO_2 (Autotrophs)	H_2O, H_2S, or organic molecules	Cyanobacteria, Purple sulfur bacteria
Chemoautotrophs	Chemical reactions	CO_2	Reduced inorganic compounds (NH_3, H_2, Fe_2^{+})	Nitrifying bacteria, Methanogens
Chemoheterotrophs	Organic compounds	Organic compounds	Organic molecules (glucose, acetate)	E. coli, Fungi

Photoheterotrophs	Light	Organic compounds	Organic or inorganic compounds	Purple non-sulfur bacteria

Micronutrients

Micronutrients are required in trace amounts but are equally important for microbial metabolism.

- **Iron (Fe)**: Essential for electron transport chains and enzymes like cytochromes. Microorganisms often produce siderophores to scavenge iron from their environment.
- **Manganese (Mn), Zinc (Zn), Copper (Cu), Molybdenum (Mo), and Cobalt (Co)**: These trace metals serve as cofactors in enzymatic reactions and are crucial for maintaining microbial cellular processes.

Growth Factors

- **Vitamins**: Many microorganisms require vitamins, which act as coenzymes in metabolic reactions. For example, *Vitamin B12* is crucial for some bacteria, and *biotin* is required for the synthesis of fatty acids.
- **Amino Acids**: Certain microorganisms cannot synthesize all of the 20 amino acids and thus require some to be supplied from the environment (auxotrophs).
- **Other growth Factors:** Some microorganisms require specific organic compounds such as vitamins, amino acids, or purines/pyrimidines that they cannot synthesize.

3. Environmental Factors Influencing Nutrient Availability

- **Soil and Water Microbes**: The availability of nutrients in soils and water is influenced by factors such as pH, temperature, organic matter, and oxygen availability. Microorganisms in soils often rely on decomposing organic material, while aquatic microorganisms may depend on the availability of nutrients like nitrate and phosphate from runoff or natural processes.
- **Nutrient Cycling**: The cycling of nutrients in ecosystems is driven by microorganisms. Nitrogen cycling, for example, involves processes such as nitrogen fixation, nitrification, denitrification, and ammonification, where different groups of microbes play different roles in converting nitrogen between forms.

4. Adaptations to Nutrient Limitation

Microorganisms have developed various strategies to survive and thrive in nutrient-limited environments:

- **Siderophore Production**: To acquire iron in iron-limited environments, many microorganisms produce siderophores—small molecules that bind to iron and facilitate its uptake.
- **Biofilm Formation**: In environments where nutrients are patchy, microorganisms often form biofilms, which are communities of cells encased in a self-produced

matrix. Biofilms allow microorganisms to retain nutrients and protect themselves from environmental stresses.

- **Metabolic Flexibility**: Some microorganisms are capable of shifting their metabolic pathways based on nutrient availability. For example, *Pseudomonas* species can switch between aerobic and anaerobic respiration depending on oxygen levels.
- **Spore Formation**: In nutrient-deprived conditions, some bacteria (e.g., *Bacillus* and *Clostridium*) can form spores, which are highly resistant structures that can survive extreme environmental conditions until more favorable conditions return.

5. Microbial Ecology and Nutrient Competition

In natural environments, microorganisms often compete for limited resources. This competition can lead to the evolution of unique metabolic strategies, such as:

- **Symbiosis**: Symbiotic relationships, including mutualism and commensalism, can help microorganisms access nutrients. For example, *Rhizobium* bacteria provide nitrogen to plants, while receiving carbon compounds in return.
- **Antibiotic Production**: Some microorganisms produce antibiotics as a means to outcompete others for resources. This phenomenon is observed in soil bacteria such as *Streptomyces*, which produce antibiotics to inhibit the growth of other microorganisms.

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Lec.2

Introduction to Food Media in Biotechnology

Food media are essential nutrient-rich substances that support the growth and proliferation of microbial and cellular cultures in biotechnology. These media serve as the primary source of nutrients, energy, and growth factors required for various biotechnological applications, including industrial fermentation, pharmaceuticals, food production, and synthetic biology. The composition of food media significantly impacts microbial physiology, metabolic pathways, and overall productivity.

There are several types of food media, each designed for specific applications:

- **Solid Media:** Agar-based media commonly used for microbial isolation, screening, and morphological characterization.
- **Liquid Media:** Used for large-scale fermentation processes, ensuring uniform nutrient distribution and easy metabolic by product removal.
- **Semi-solid Media:** These are specialized media with a gel-like consistency, used for microbial motility studies and anaerobic bacterial growth.

Food media must be carefully formulated to maintain osmotic balance, supply adequate nutrients, and prevent microbial contamination. The optimization of these media is essential for achieving high-yield and high-purity bioproducts (**Fig. 4**).

Figure 4: Diagram illustrating different types of culture media

Optimization of Food Media

To maximize the efficiency of food media in biotechnology, several critical parameters must be controlled:

- **pH and Temperature Control:** Each microbial species has an optimal pH and temperature range for growth. For example, most bacteria thrive at a pH of 6.5–7.5, while fungi prefer slightly acidic conditions (pH 4–5). Temperature regulation is also crucial, as deviations can lead to enzyme denaturation or reduced metabolic activity (Fig.5).

- **Aeration and Oxygen Supply:** Oxygen availability directly impacts microbial respiration and metabolite production. Aerobic cultures require effective oxygen transfer, which is achieved through agitation and controlled aeration in bioreactors.
- **Nutrient Balance:** Ensuring the right proportion of carbon, nitrogen, and micronutrients is essential for sustained microbial growth and product formation.
- **Use of Additives:** Certain compounds such as surfactants, inducers, and co-factors can enhance microbial metabolism, improve biofilm formation, and increase product yield.

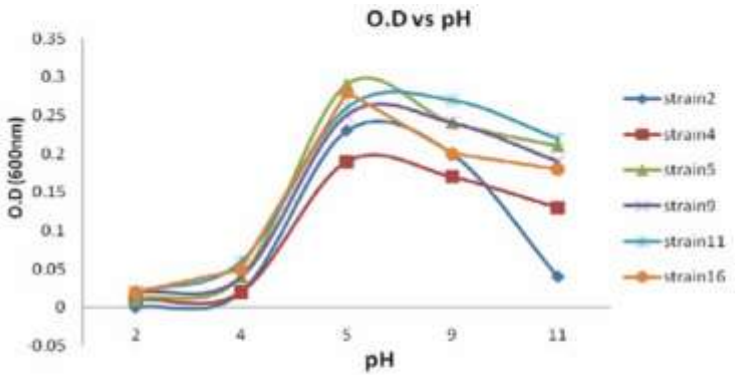

Figure 5: Diagram illustrating different degree of pH for bacterial growth

Industries Benefiting from Food Media

Food media are widely used in several industrial applications, including:

- **Fermentation Industry:** Used in large-scale production of antibiotics (penicillin, streptomycin), industrial enzymes (amylases, proteases), and bioactive compounds.
- **Food Industry:** Essential for dairy fermentation (yogurt, cheese), alcoholic beverage production (beer, wine), and probiotic formulations that enhance gut health.
- **Pharmaceutical Industry:** Supports recombinant microbial cultures for vaccine production, as well as cell culture applications in tissue engineering and regenerative medicine.
- **Biofuel Production:** Optimized media formulations are crucial for cost-effective production of bioethanol and biodiesel from microbial fermentation (Fig.6).

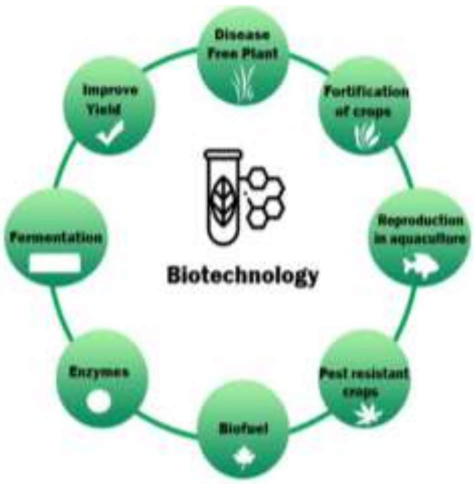

Figure 6: Flowchart showing the role of food media in various biotechnological applications.

Enriching Media for Specialized Cultures
Types of Specialized Media

Specialized media are formulated to support specific microbial growth requirements and differentiation:

- **Selective Media:** These media promote or inhibit specific microorganisms. For instance, MacConkey agar selectively grows Gram-negative bacteria while inhibiting Gram-positive species.
- **Differential Media:** Allows distinction between microbial species based on metabolic activity. Blood agar, for example, differentiates bacteria based on hemolysis patterns.
- **Enrichment Cultures:** These media support the growth of specific microbes from complex environmental samples by providing targeted nutrients (Fig.7).

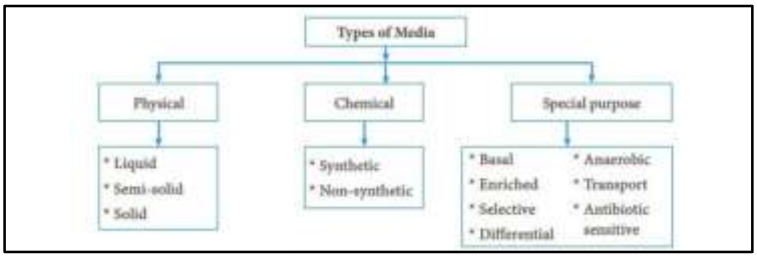

Figure 7: Flowchart showing different specialized media and their applications.

Sustainable and Cost-Effective Media Development
Eco-friendly and Cost-efficient Alternatives

Given the rising costs of industrial-scale biotechnology, researchers are exploring sustainable and cost-effective media alternatives:

- **Use of Agro-Industrial Waste:** Byproducts such as molasses, whey, and spent grains serve as economical carbon sources. Corn steep liquor is a rich nitrogen source that can replace expensive synthetic compounds.
- **Bioreactor Optimization:** The design and operation of bioreactors play a key role in reducing production costs while maximizing microbial growth efficiency.
- **Alternative Protein and Carbohydrate Sources:** Exploring novel sustainable inputs like algae-derived proteins and insect-based carbohydrate sources is an emerging trend (Table 2).

Table 2: Comparative analysis of synthetic vs. natural media in terms of cost-effectiveness and sustainability

	Natural Biodegradable Polymers	Synthetic Biodegradable Polymers
Source	Renewable biological sources (plants, animals, microorganisms)	Synthetic processes, renewable feedstocks, sometimes petroleum-based
Examples	Cellulose, chitosan, collagen, gelatin	PLA, PCL, PHAs, polyanhydrides, PVA
Properties	Biocompatible, nontoxic, lower mechanical strength, and thermal stability	Customizable properties, consistent quality, tailored mechanical, and thermal properties
Production Methods	Extraction, purification, chemical, or enzymatic modification	Polymerization (ring-opening, polycondensation), bacterial fermentation
Applications	Food packaging, medical applications, agriculture, textiles	Packaging, medical devices, drug delivery, agriculture, consumer goods
Advantages	High biocompatibility, nontoxicity, renewable sources, easier biodegradation	Customizable, consistent quality, wide range of applications
Disadvantages	Quality variability, limited property customization, potential allergenicity	Higher production costs, potential use of toxic intermediates, specific biodegradation conditions
Biodegradability	Often readily biodegradable due to natural	Biodegradability depends on envi-

Future Trends in Food Media Biotechnology

The future of food media biotechnology is driven by advanced technologies and novel bioprocessing strategies:

- **Synthetic and Defined Media:** Precision-controlled nutrient formulations tailored for specific microbial applications (Fig.8).
- **CRISPR and Genetic Engineering in Microbial Growth Enhancement:** Engineered microbes with improved nutrient uptake and metabolic efficiency are being developed for industrial applications.
- **AI and Machine Learning:** Data-driven media optimization techniques are revolutionizing yield prediction and cost minimization in fermentation processes.
- **Personalized Nutrition:** Custom-designed probiotic formulations based on individual gut microbiomes represent a promising direction in health biotechnology.

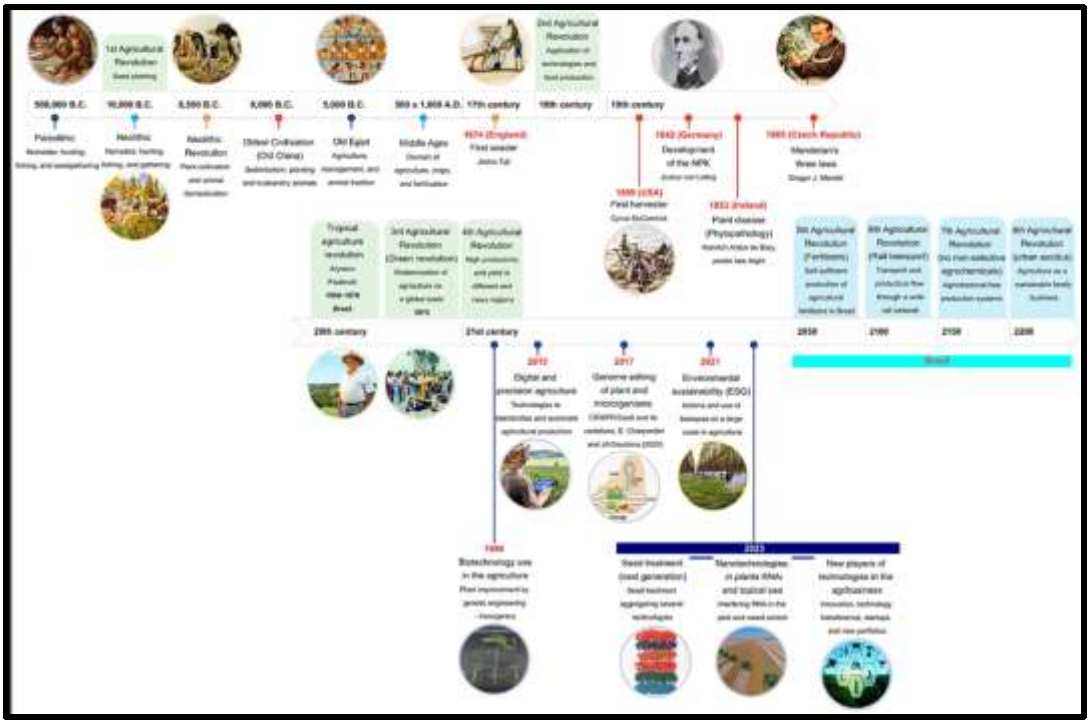

Figure 8: Timeline predicting future developments in food media biotechnology .

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Principles of Biotechnology II

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Lec.3

Antibiotics and Microorganisms Used in Production

Introduction to Antibiotics

Antibiotics are chemical substances produced by microorganisms that inhibit the growth of or kill other microorganisms. They are widely used in medicine, agriculture, and research to control bacterial infections and enhance productivity.

1. Definition and History of Antibiotics

- Antibiotics are natural, semi-synthetic, or synthetic compounds that target bacterial infections.
- Discovered by Alexander Fleming in 1928 with the discovery of penicillin from *Penicillium notatum*.
- Development of antibiotics revolutionized medicine, reducing mortality from bacterial infections.

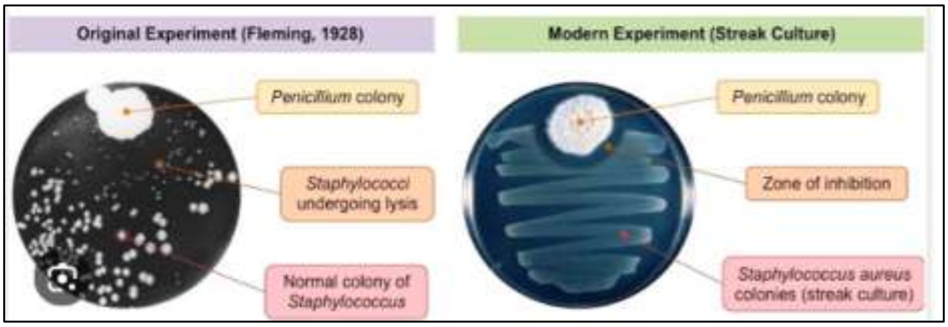

Figure 1: Alexander Fleming and the Discovery of Penicillin

2. Classification of Antibiotics

Antibiotics can be classified based on their source, spectrum of activity, and mode of action.

A. Based on Origin

1. **Natural antibiotics** - Derived from microorganisms (e.g., Penicillin from fungi, Streptomycin from bacteria).
2. **Semi-synthetic antibiotics** - Modified natural antibiotics (e.g., Amoxicillin, Cefuroxime).
3. **Synthetic antibiotics** - Fully synthesized in laboratories (e.g., Sulfonamides, Fluoroquinolones).

B. Based on Spectrum of Activity

1. **Broad-spectrum antibiotics** - Effective against both Gram-positive and Gram-negative bacteria (e.g., Tetracycline, Chloramphenicol).
2. **Narrow-spectrum antibiotics** - Effective against specific bacterial groups (e.g., Penicillin G for Gram-positive bacteria).

C. Based on Mechanism of Action

1. **Inhibitors of cell wall synthesis** (e.g., Penicillins, Cephalosporins)
2. **Protein synthesis inhibitors** (e.g., Tetracyclines, Macrolides)
3. **DNA/RNA synthesis inhibitors** (e.g., Fluoroquinolones, Rifampin)
4. **Metabolic pathway inhibitors** (e.g., Sulfonamides)

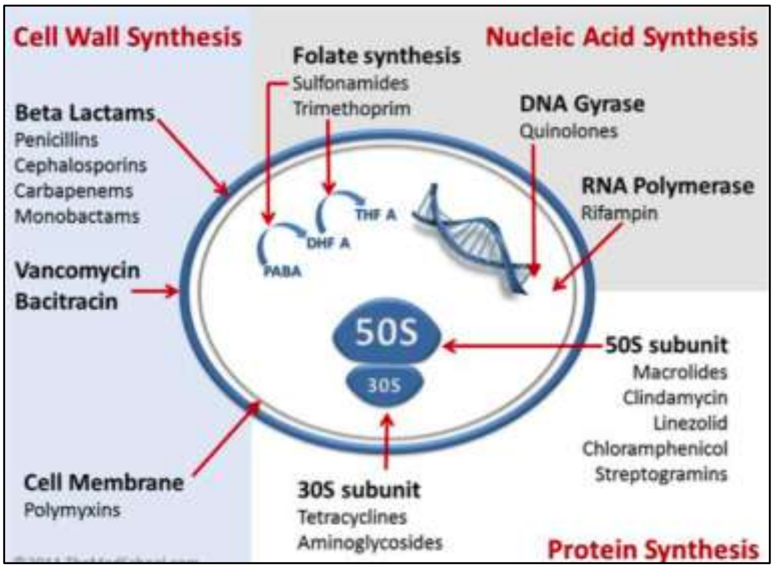

Figure 2: Classification of Antibiotics Based on Mode of Action

Microorganisms Used in Antibiotic Production

Several microorganisms, including bacteria and fungi, play a crucial role in the biosynthesis of antibiotics.

3. Fungal Sources of Antibiotics

A. Penicillium *spp.*

- Produces **Penicillin**, the first discovered antibiotic.
- Inhibits bacterial cell wall synthesis, making it effective against Gram-positive bacteria.

B. Acremonium *(formerly* Cephalosporium*) spp.*

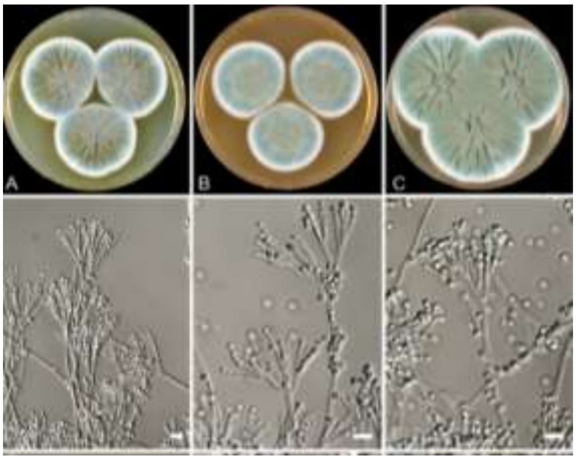

Figure 3: *Penicillium* Fungus Producing Penicillin

- Produces **Cephalosporins**, structurally related to penicillin but broader in spectrum.

4. Bacterial Sources of Antibiotics

A. Streptomyces *spp. (Actinobacteria)*

- Responsible for the majority of naturally derived antibiotics.
- Produces:
    - **Streptomycin** (used against tuberculosis)
    - **Tetracyclines** (broad-spectrum antibiotic)
    - **Chloramphenicol** (inhibits bacterial protein synthesis)

B. Bacillus *spp.*

- Produces:
    - **Bacitracin** (effective against Gram-positive bacteria)
    - **Polymyxins** (targets Gram-negative bacteria)

5. Industrial Production of Antibiotics

The large-scale production of antibiotics involves several steps:

A. Strain Selection and Genetic Improvement

- Selection of high-yielding microbial strains.
- Genetic engineering to enhance antibiotic production.

B. Fermentation Process

- **Batch Fermentation**: Microorganisms grow in a controlled environment to maximize antibiotic yield.
- **Continuous Fermentation**: Constant production with fresh nutrients added and antibiotics harvested regularly.

C. Downstream Processing

- **Filtration and Extraction**: Removes microbial cells and isolates the antibiotic.
- **Purification and Formulation**: The antibiotic is purified, crystallized, and formulated into usable forms (tablets, injections, etc.).

6. Challenges and Future Perspectives

A. Antibiotic Resistance

- Overuse and misuse of antibiotics have led to resistance (e.g., MRSA, multidrug-resistant *E. coli*).
- Need for new antibiotics and alternative treatments.

B. Biotechnological Advancements

- Genetic modification of microbes to enhance antibiotic production.
- Synthetic biology for novel antibiotics.
- Phage therapy as an alternative to antibiotics.

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Lec.4

Genetics, Genetic Engineering, and Categorical Enzymes

Introduction to Genetics

Genetics is the branch of biological science concerned with the study of genes, genetic variation, and heredity. It is a foundational discipline that explains how traits are transmitted from one generation to the next and the molecular mechanisms governing these processes. Over the past century, the field of genetics has undergone significant advancements, driven by groundbreaking discoveries that have deepened our understanding of heredity and biological diversity. The application of genetic knowledge extends across various fields, including medicine, agriculture, and biotechnology. By deciphering genetic information, researchers have been able to develop targeted therapies for genetic disorders, improve crop yields through genetic modification, and enhance industrial processes through bioengineering. As scientific knowledge in genetics continues to evolve, its implications in medicine, agriculture, and industry are expected to grow exponentially, shaping the future of human health and sustainability.

1. Historical Overview of Genetics

The origins of genetics as a scientific discipline can be traced back to the pioneering work of Gregor Mendel in the 19th century. Mendel's experiments with pea plants established the foundational principles of inheritance, including the laws of segregation and independent assortment. Despite the significance of his findings, Mendel's work remained largely unrecognized until the early 20th century when the principles of classical genetics were rediscovered and integrated into modern biology. Subsequent discoveries, such as the identification of DNA as the genetic material and the elucidation of its double-helix structure by James Watson and Francis Crick in 1953, further propelled the field forward. The completion of the Human Genome Project in 2003 marked a major milestone in genetics, enabling scientists to map the entire human genome and paving the way for revolutionary advancements in genetic engineering and precision medicine. Today, genetic research continues to push the boundaries of knowledge, offering insights into hereditary diseases, evolutionary biology, and potential applications in genetic therapy.

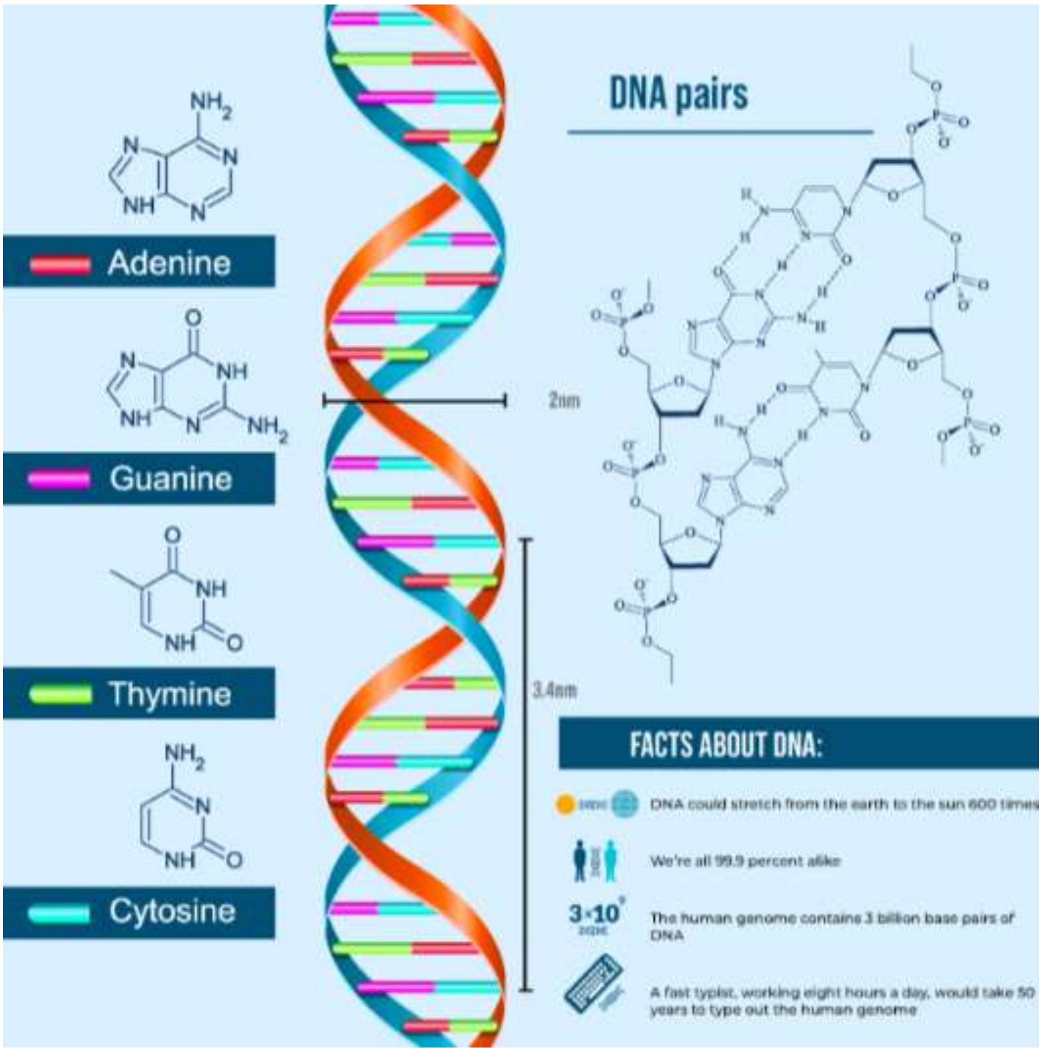

Figure 1: DNA Structure

2. The Central Dogma of Molecular Biology

The central dogma of molecular biology describes the fundamental flow of genetic information within living organisms. It outlines the three key processes that govern the expression of genetic material: DNA replication, transcription, and translation. DNA replication ensures that genetic material is faithfully copied before cell division, preserving genetic continuity across generations. Transcription is the process by which DNA is transcribed into messenger RNA (mRNA), which serves as an intermediary molecule carrying genetic instructions from the nucleus to the ribosomes. Translation, the final step, involves the decoding of mRNA sequences into functional proteins that perform vital cellular functions. Each of these processes is crucial for maintaining the integrity of genetic information and facilitating cellular development. Any disruption in these mechanisms can lead to genetic mutations, potentially resulting in hereditary disorders or diseases such as cancer. A deep understanding of these molecular processes is essential for advancements in genetic research, biotechnology, and therapeutic interventions.

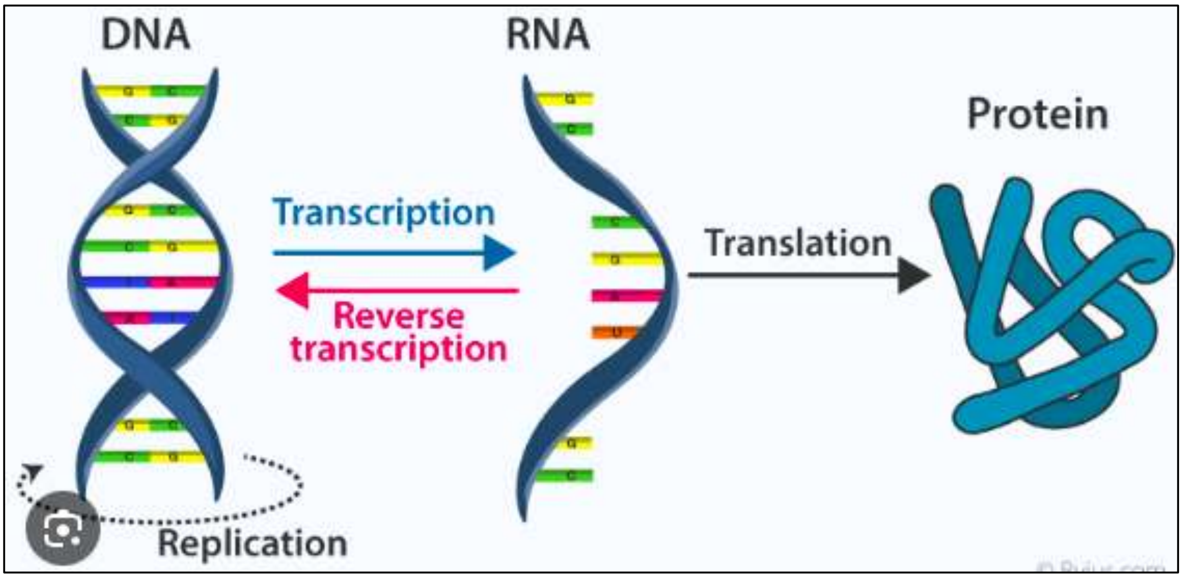

Figure 2: Central Dogma of Molecular Biology

Genetic Engineering: Principles and Applications

Genetic engineering, also known as genetic modification, involves the direct manipulation of an organism's genome to introduce, alter, or remove specific genetic traits. This cutting-edge field has rapidly transformed numerous industries, offering innovative solutions in medicine, agriculture, and biotechnology. Genetic engineering techniques enable scientists to enhance disease resistance in crops, develop gene therapies for hereditary disorders, and produce bioengineered pharmaceuticals. However, despite its immense potential, genetic engineering raises ethical and safety concerns that necessitate careful regulation and responsible implementation.

3. Techniques in Genetic Engineering

Several sophisticated methodologies have been developed to manipulate genetic material effectively. Recombinant DNA technology involves the combination of DNA from different sources to create genetically modified organisms (GMOs) with improved traits. This technique has wide-ranging applications in medical research, agriculture, and industrial biotechnology. CRISPR-Cas9, a revolutionary gene-editing tool, allows precise modifications of DNA sequences, holding promise for correcting genetic disorders at the molecular level. Gene cloning techniques enable the replication of specific genes for research, therapeutic, or agricultural purposes. The polymerase chain reaction (PCR) is another fundamental technique used to amplify DNA sequences, facilitating genetic studies and forensic applications. These techniques have transformed genetic research, providing powerful tools for studying gene function, developing new therapies, and enhancing agricultural productivity.

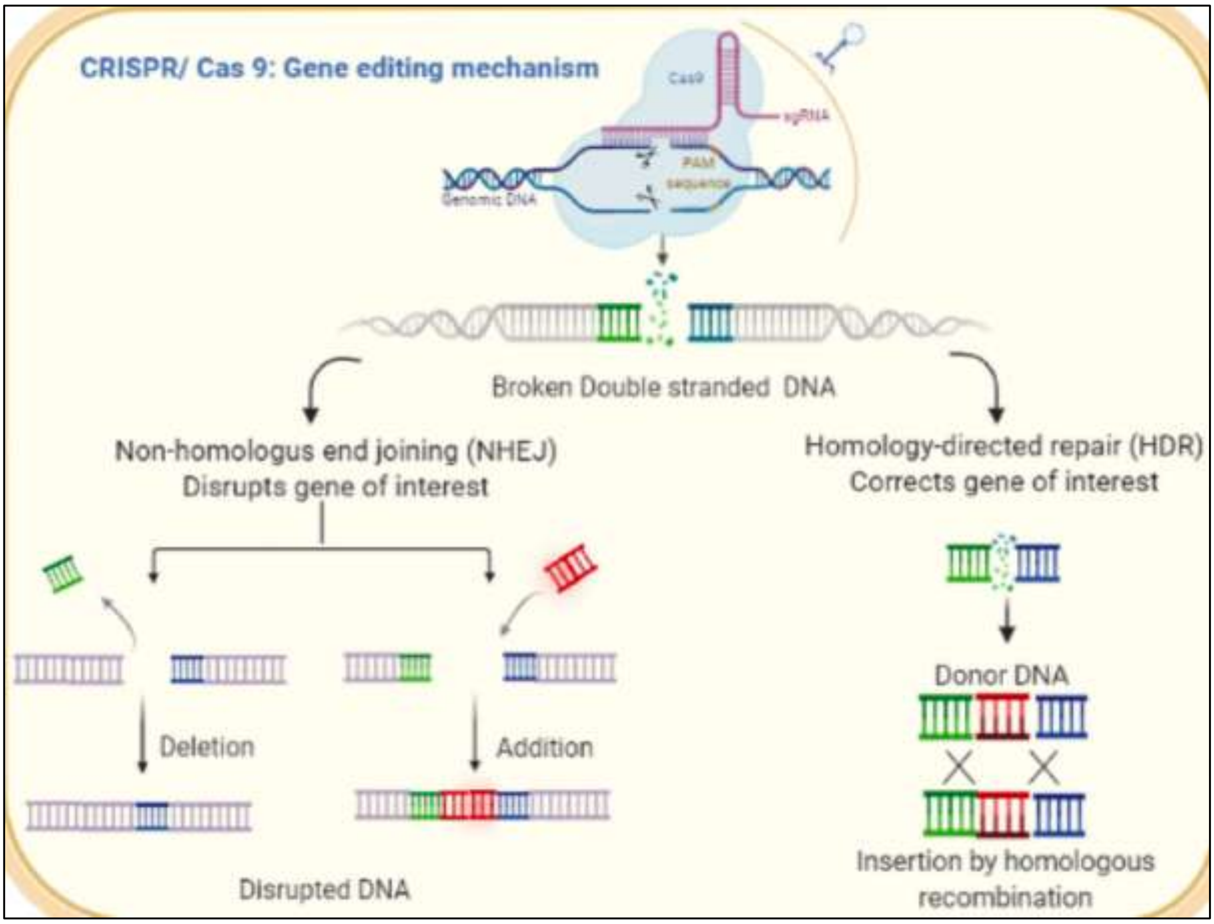

Figure 3: CRISPR Gene Editing Mechanism

4. Applications of Genetic Engineering

Genetic engineering has revolutionized numerous fields, offering transformative applications across medicine, agriculture, and industry. In medicine, gene therapy has emerged as a promising approach for treating genetic disorders by repairing or replacing defective genes. The development of genetically modified bacteria has enabled the large-scale production of insulin, growth hormones, and other therapeutic proteins. In agriculture, genetically modified crops have been engineered to increase yield, enhance resistance to pests and diseases, and improve nutritional content, contributing to global food security. In industrial applications, genetic engineering is utilized for enzyme production, biofuel development, and bioremediation, fostering environmental sustainability. While the benefits of genetic engineering are substantial, it is imperative to address ethical, regulatory, and safety concerns to ensure responsible and equitable use of this technology.

7. References

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Lec.5

The concept of enzymes and microorganisms producing enzymes and their industrial andmedical applications, Industrial production of enzymes

- **Introduction**

Enzymes are biological macromolecules that function as catalysts, accelerating chemical reactions essential for life. They are primarily composed of proteins, though some RNA molecules (ribozymes) also exhibit catalytic properties. Enzymes play a critical role in metabolism, digestion, DNA replication, and numerous other biological processes, ensuring that reactions occur at rates necessary for cellular function.

Importance of Enzymes in Biological Systems

Enzymes are fundamental to sustaining life because they:

- **Reduce Activation Energy**: They speed up reactions by lowering the energy barrier required for reactions to proceed.
- **Enable Metabolic Pathways**: Without enzymes, essential metabolic reactions would be too slow to support life.
- **Ensure Specificity**: Each enzyme is highly specific, catalyzing only one type of reaction or acting on a particular substrate.
- **Allow Regulation**: Enzymes can be activated or inhibited to control metabolic pathways and maintain homeostasis.

Structure and Function of Enzymes

Enzymes possess an active site where substrate binding occurs, facilitating the reaction (Fig.1). This specificity is often explained by:

- **Lock and Key Model**: The substrate fits precisely into the enzyme's active site.
- **Induced Fit Model**: The enzyme undergoes conformational changes to accommodate the substrate.

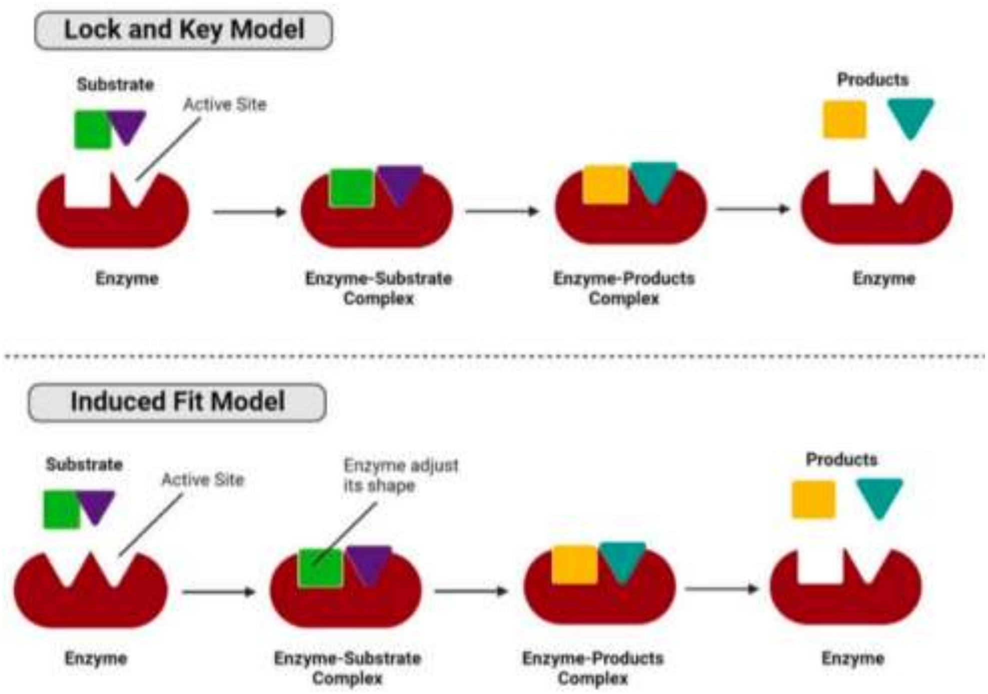

Fig 1: **Structure of Enzymes**

Enzymes can be categorized based on their function:

1. **Oxidoreductases** - Involved in oxidation-reduction reactions (e.g., catalase).
2. **Transferases** - Transfer functional groups between molecules (e.g., kinases).
3. **Hydrolases** - Catalyze hydrolysis reactions (e.g., proteases, lipases).
4. **Lyases** - Break bonds without hydrolysis or oxidation (e.g., decarboxylases).
5. **Isomerases** - Convert molecules into their isomers (e.g., phosphoglucoisomerase).
6. **Ligases** - Join two molecules together (e.g., DNA ligase).

Factors Affecting Enzyme Activity

1. **Temperature**: Enzymes have an optimal temperature range; excessive heat denatures them, while low temperatures slow down reactions.
2. **pH**: Each enzyme functions best at a specific pH (e.g., pepsin in the stomach at pH 2, amylase in saliva at pH 7).
3. **Substrate Concentration**: Increasing substrate levels enhances reaction rate until saturation is reached.
4. **Enzyme Concentration**: More enzyme molecules increase reaction speed.
5. **Inhibitors**:
   - **Competitive Inhibition**: Inhibitors compete with the substrate for the active site.
   - **Non-Competitive Inhibition**: Inhibitors bind to another site, altering enzyme shape.

Microorganisms as Sources of Enzymes

Microbial enzyme production is preferred due to advantages such as rapid growth, ease of genetic modification, and high enzyme yield (Table 1).

Table1 : Common Microbial Sources and Their Functions

Enzyme	Microorganism	Function
Amylase	*Bacillus subtilis*, *Aspergillus niger*	Breakdown of starch into sugars
Protease	*Bacillus licheniformis*, *Aspergillus* species	Protein hydrolysis in detergents & food
Lipase	*Candida rugosa*, *Pseudomonas* species	Fat digestion, dairy processing
Cellulase	*Trichoderma reesei*	Decomposing plant fibers, biofuel production

Laccase	*Trametes versicolor*	Textile dye degradation, bioremediation

Industrial Applications of Enzymes

1. **Food Industry**:
   - **Brewing**: Amylases break down starch into fermentable sugars for alcohol production.
   - **Cheese Making**: Rennet enzymes assist in milk coagulation.
   - **Meat Tenderization**: Proteases like papain soften meat fibers.
2. **Detergent Industry**:
   - Proteases remove protein stains (e.g., blood, sweat).
   - Lipases break down fats and grease.
3. **Textile and Leather Industry**:
   - Cellulases improve fabric texture.
   - Proteases aid in leather processing.
4. **Paper Industry**:
   - Xylanases enhance paper bleaching, reducing chemical usage.
5. **Biofuel Production**:
   - Enzymes decompose biomass into fermentable sugars for ethanol production.

Medical Applications of Enzymes

1. **Diagnostics**:
   - **Glucose Oxidase**: Used in diabetes monitoring (glucose test strips).
   - **Urease**: Assists in kidney function assessment.
2. **Therapeutics**:

- **Streptokinase**: Dissolves blood clots in stroke and heart attack patients.
- **Asparaginase**: Used in leukemia treatment by depleting asparagine needed by cancer cells.

3. **Pharmaceutical Applications**:
   - Enzymes aid in drug synthesis (e.g., antibiotics like penicillin).
   - DNA polymerases assist in PCR for genetic testing.

Industrial Production of Enzymes

Process Overview

1. **Selection of Microorganism**:
   - Screening for strains that produce high enzyme yields.
   - Genetic engineering can enhance production.
2. **Fermentation**:
   - Large-scale cultivation in bioreactors under optimal conditions.
   - **Submerged Fermentation (SmF)**: Enzyme production in liquid media.
   - **Solid-State Fermentation (SSF)**: Uses solid materials like bran or agricultural waste.
3. **Downstream Processing**:
   - **Cell Harvesting**: Filtration or centrifugation to collect microbial cells.
   - **Enzyme Extraction**: Breaking cells to release enzymes.
   - **Purification**: Precipitation, chromatography, or ultrafiltration.
4. **Formulation & Storage**:
   - Enzymes are stabilized via freeze-drying or encapsulation.

Graph: Enzyme Production Process

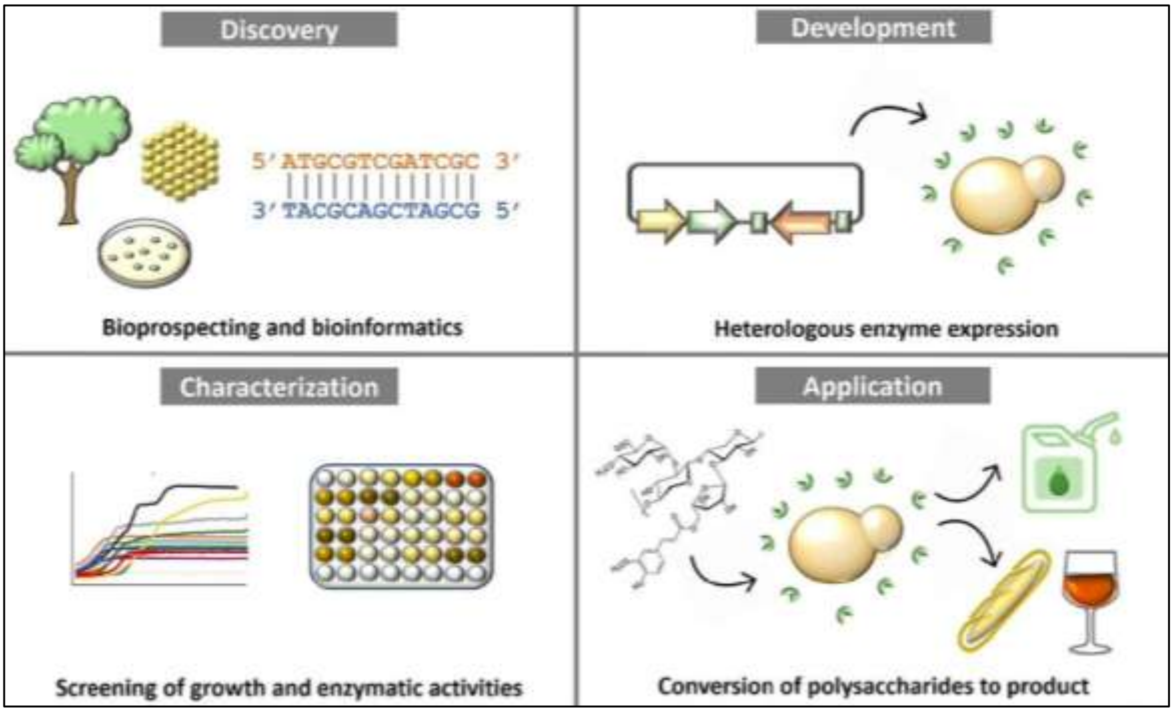

Figure 2: A typical enzyme production process includes:

1. **Microbial Strain Selection** → 2. **Fermentation in Bioreactor** → 3. **Enzyme Extraction & Purification** → 4. **Product Formulation & Quality Control**

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Lec.6

Restriction Enzymes - Concepts, Methods, and Applications

1. Introduction

Restriction enzymes, also known as restriction endonucleases, are specialized proteins that cut DNA at specific sequences. These enzymes play a crucial role in genetic engineering, molecular biology, and biotechnology. Their discovery revolutionized DNA manipulation, enabling advancements in recombinant DNA technology, genetic mapping, and forensic science. The ability of these enzymes to recognize and cleave specific DNA sequences has provided researchers with powerful tools to modify genetic material with precision.

2. Types of Restriction Enzymes

Restriction enzymes are categorized into three main types based on their recognition sequence and cutting mechanism. Each type has distinct properties and applications in molecular biology (Table 2).

Table 2: Types of Restriction Enzymes

Type	Recognition Site	Cutting Mechanism	Example Enzymes
Type I	Random sites far from recognition sequence	Cuts at distant locations, requires ATP	EcoKI
Type II	Specific sequences within the recognition site	Cuts at defined positions	EcoRI, HindIII
Type III	Short distance away from the recognition site	Requires ATP, cuts at fixed positions	EcoP15I

Type I enzymes are complex, requiring ATP and other cofactors for DNA cleavage, making them less commonly used in laboratory settings. Type II enzymes are the most widely used due to their ability to produce predictable and consistent cuts. Type III enzymes exhibit intermediate complexity, cutting DNA at short distances away from their recognition site.

3. **Recognition and Cutting Mechanism**

3.1 Palindromic Recognition Sequences

Most Type II restriction enzymes recognize palindromic sequences, meaning the sequence reads the same forward and backward on complementary strands. This palindromic nature allows for precise cleavage and facilitates DNA recombination.

Example of a Palindromic Recognition Site

EcoRI recognition site: 5’-GAATTC-3’
Cut Site: 5’-G↓AATTC-3’

The presence of palindromic sequences in DNA allows enzymes to bind symmetrically and introduce breaks that are crucial for cloning and recombination experiments.

3.2 Types of Cuts

Restriction enzymes produce two main types of DNA cuts (Figure 3):

- **Sticky Ends:** Creates overhangs, useful for DNA ligation (e.g., EcoRI). These ends enhance recombination by allowing complementary base pairing between different DNA fragments.
- **Blunt Ends:** Creates even cuts, useful in cloning (e.g., HaeIII). Although blunt ends do not facilitate efficient recombination, they are essential in

certain genetic modifications requiring precise joining of DNA fragments.

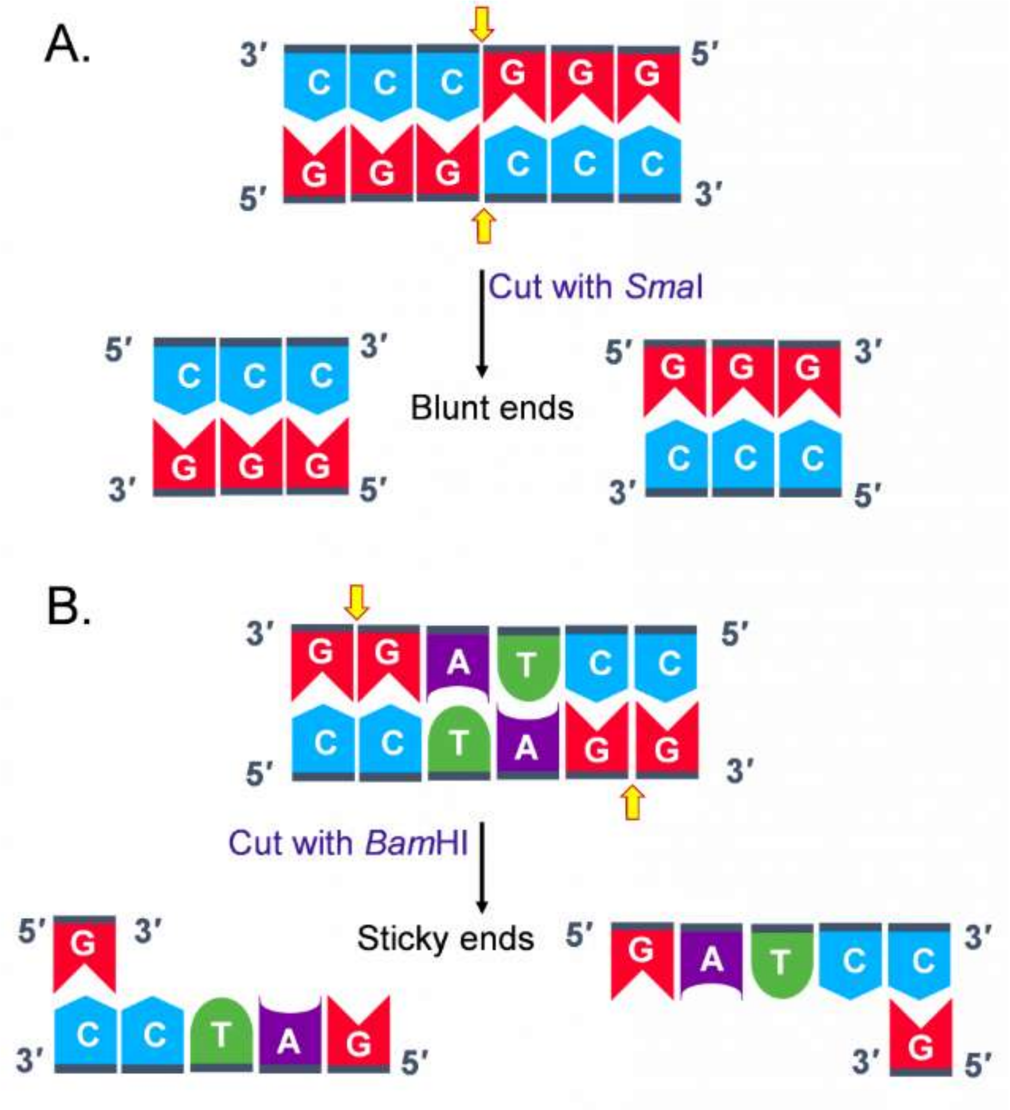

Fig. 2: Types of DNA Cuts

4. Methods of Restriction Digestion

Restriction digestion is the process of cutting DNA with restriction enzymes. The method involves:

1. **Preparation of DNA sample** (e.g., plasmid or genomic DNA). DNA must be purified and free of contaminants such as proteins and salts.
2. **Selection of appropriate restriction enzyme** based on the target sequence.

3. **Incubation with buffer and enzyme at optimal temperature** (usually 37°C). The buffer composition is crucial as it ensures enzyme activity and stability.
4. **Termination of reaction** (by heat inactivation or chemical methods). Heat inactivation (e.g., at 65°C for 10 minutes) denatures the enzyme and stops further digestion.
5. **Analysis of digested DNA** (using gel electrophoresis). This step confirms successful digestion by visualizing DNA fragments under UV light after staining with ethidium bromide or SYBR Green.

Table 2: Common Restriction Enzymes and Their Properties

Enzyme	Recognition Sequence	Cut Type	Application
EcoRI	5’-GAATTC-3’	Sticky Ends	Cloning
HindIII	5’-AAGCTT-3’	Sticky Ends	Recombinant DNA
HaeIII	5’-GGCC-3’	Blunt Ends	DNA Fingerprinting

5. Applications of Restriction Enzymes

5.1 Genetic Engineering & Cloning

Restriction enzymes allow the insertion of genes into plasmids, enabling recombinant DNA technology for gene expression studies. By cutting and pasting DNA fragments, scientists can engineer bacteria to produce proteins such as insulin, growth hormones, and vaccines.

5.2 **DNA Fingerprinting**

Used in forensic science and paternity testing by analyzing restriction fragment length polymorphisms (RFLP). This technique relies on the fact that individuals have unique DNA sequences, leading to distinct banding patterns upon digestion and electrophoresis.

5.3 **Genome Mapping**

Essential for locating genes on chromosomes and understanding genetic variations. By using multiple restriction enzymes, scientists can generate detailed maps of genomes, aiding in the study of genetic disorders and evolutionary biology.

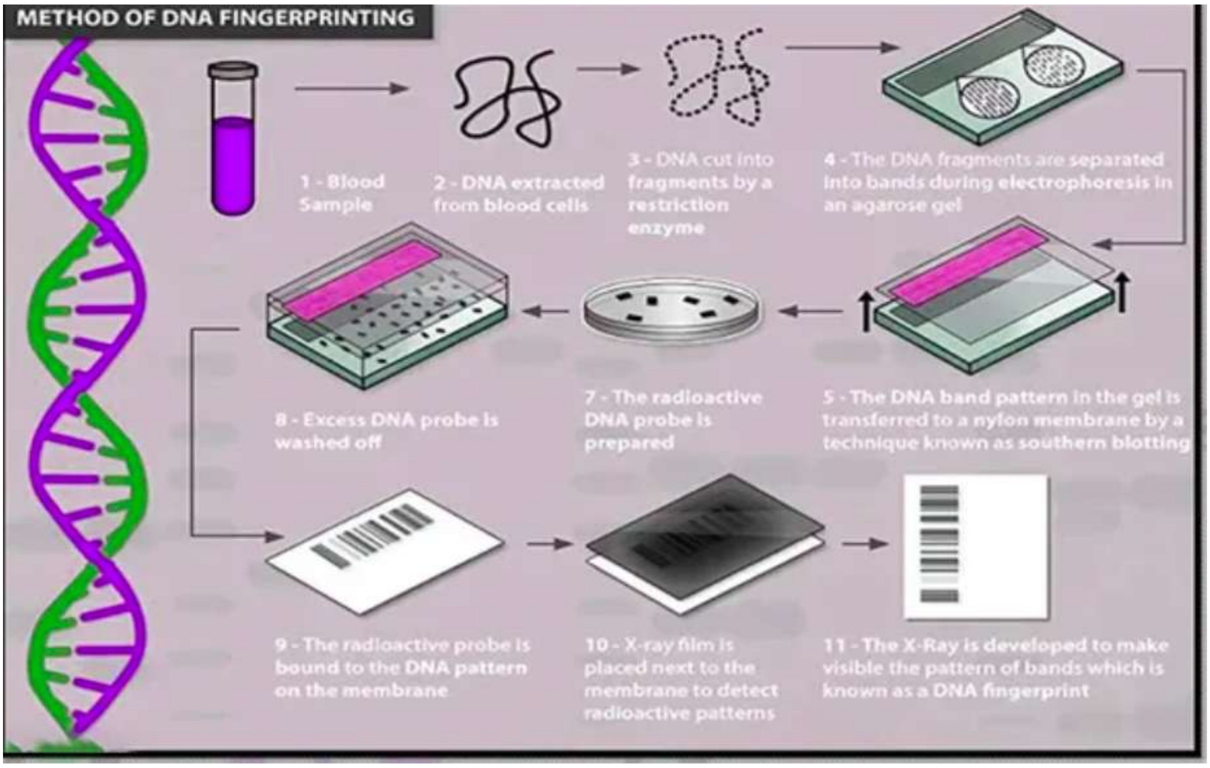

Figure 3: DNA Fingerprinting Using Restriction Digestion and Gel Electrophoresis

Illustration showing banding patterns produced by restriction digestion of DNA samples. The differences in fragment sizes reveal genetic variations among individuals.

5.4 **Molecular Diagnostics**

Used to detect mutations or genetic diseases by analyzing DNA fragment patterns after digestion. Certain mutations disrupt restriction sites, altering fragment sizes and providing a method for diagnosing genetic disorders such as sickle cell anemia and cystic fibrosis.

6. **Experimental Data and Analysis**

A graphical representation showing different fragment sizes obtained from restriction digestion of a DNA sample. By comparing banding patterns to known molecular weight markers, researchers can identify and analyze genetic sequences of interest.

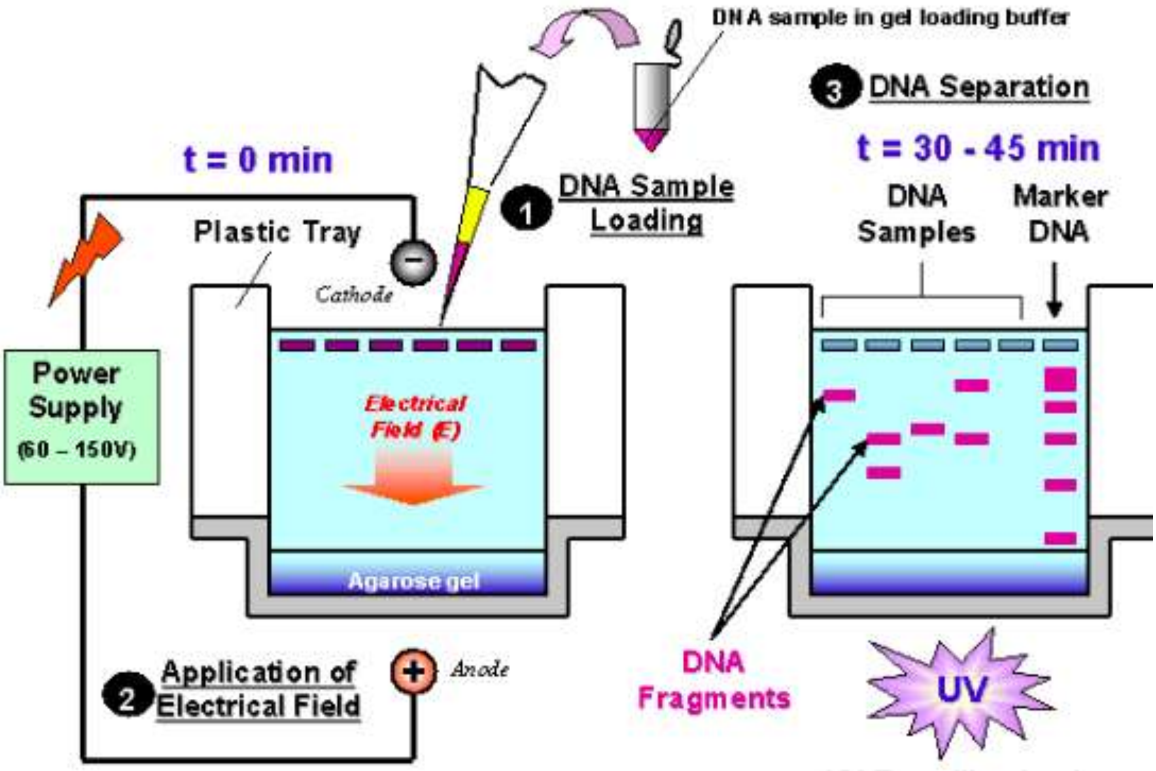

Fig4: Gel Electrophoresis of DNA Fragments After Restriction Digestion

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Principles of Biotechnology II

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Level 1
Second Semester
2024 - 2025

Lecture 7

Microbial Metabolism and Biotechnology

How the biotechnological products are produced through metabolism?

Metabolism is a constant and collective of biochemical reactions within a living organism (single or multicellular organism). The biochemical process can be classified into catabolism and anabolism. The end-products of these pathways are used for the formation of **intermediates** and **substrates** for other metabolic pathways and are known as "**metabolites**".

Products of industrial microorganisms are divided into two broad groups, those which result from primary metabolism and others which derive from secondary metabolism.

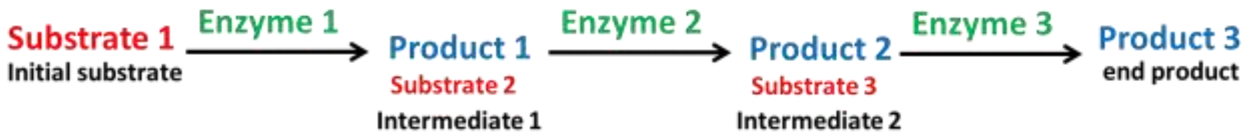
The difference between primary and secondary metabolite is ambiguous since many of the intermediates in primary metabolism is overlapping with the intermediates of secondary metabolites. Amino acids though considered a product of primary metabolite are definitely secondary metabolite too.

What is a metabolic pathway?

Metabolic pathway is the series of chemical reactions involved in converting a metabolite in the organism into a final product. A metabolic pathway is a chain of **enzymatic reactions**.

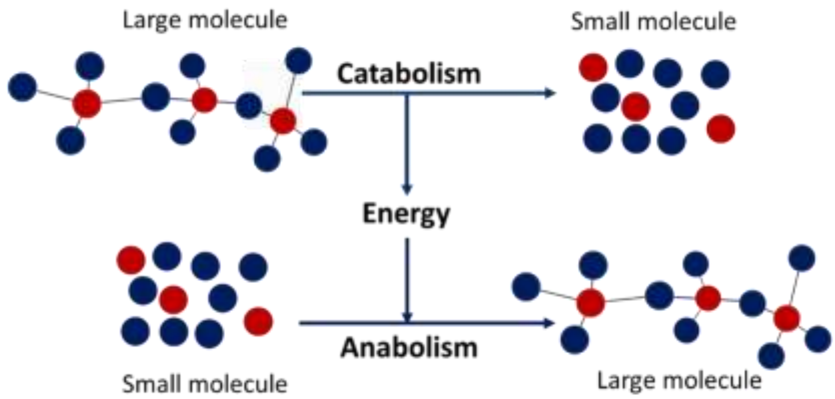
The pathway is a collection of step by step modifications:

The initial substance used as substrate by the first enzyme is transformed into a product. This product will then be the substrate for the next reaction, until the exact chemical structure necessary for the cell is reached (final product):



The compounds involved in a metabolic pathway are called **intermediates** and the final product is known as the **end-product**.

- When the series of reactions lead to the formation of a **more complex substance**, the metabolism is known as **anabolism** and the pathway an **anabolic pathway**.
- When the series of reactions lead to **less complex compounds** the metabolism is known as **catabolism**.



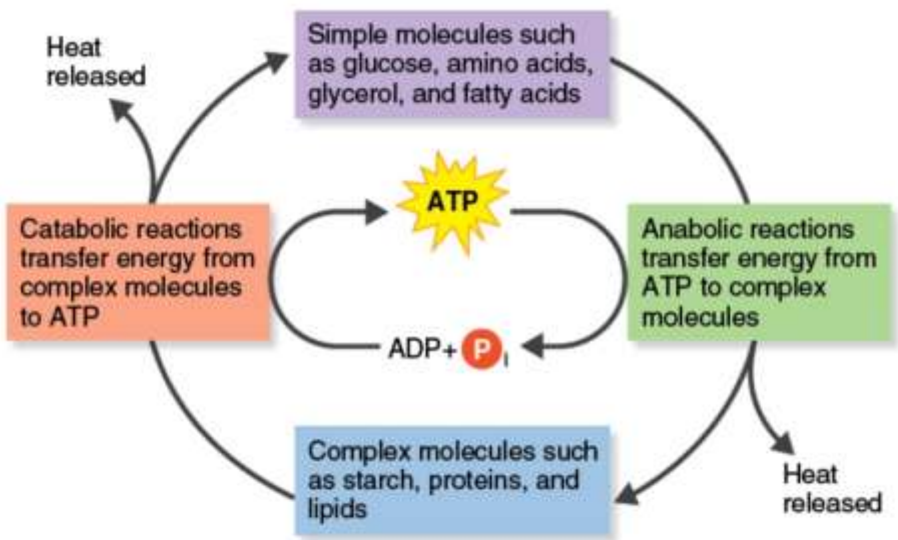


Figure. Metabolism: Relationship between anabolism and catabolism in a cell.

Breaking down glucose releases energy, which is captured by the cell in the form of **adenosine triphosphate, or ATP**. ATP **stores** energy derived from **catabolic**

reactions and releases it later to drive **anabolic reactions** and perform other cellular work.

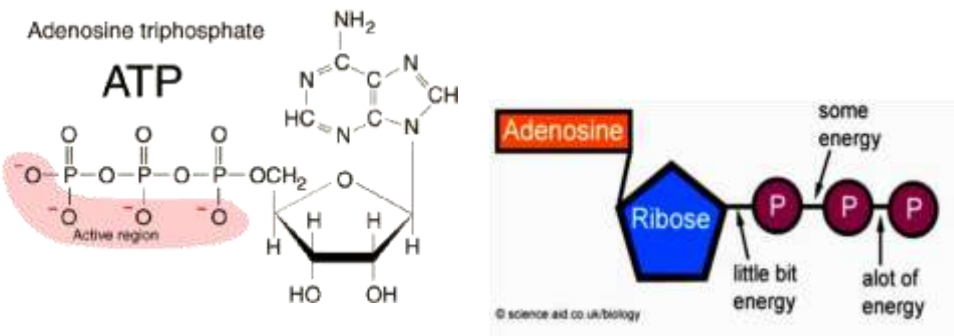
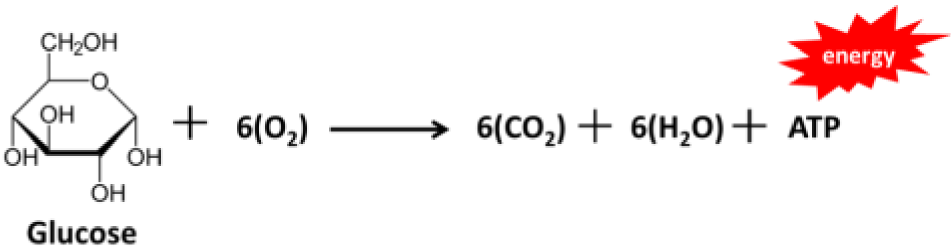


Figure. ATP molecule (ATP - an adenine, a ribose and 3 phosphate groups).

Catabolism (reactions that release energy)

Complex organic compounds are broken down into simpler units. These reactions are called catabolic or **degradative reactions**. They are generally hydrolytic reactions because they use **water** and in which chemical bonds are broken, and they are exergonic (produce more energy than they consume). For example, cells break down **sugars** into CO_2 and H_2O:



Anabolism (energy requiring reactions)

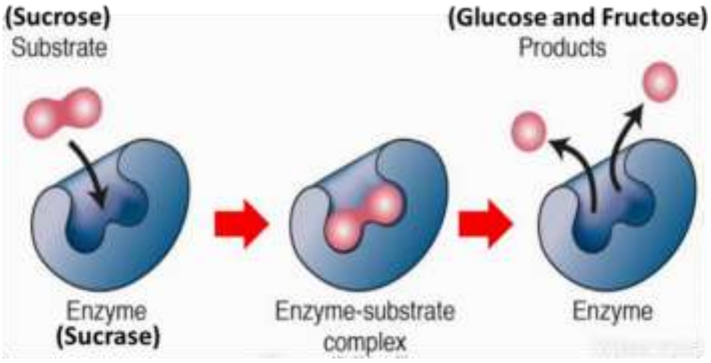
The building of complex organic molecules from simpler ones. These reactions are called anabolic or biosynthetic and they are generally dehydration synthesis reactions (reactions that release water), and they are endergonic (consume more energy than they produce). For example, formation of **proteins** from amino acids, **nucleic acids** from nucleotides, **polysaccharides** from simple sugars). These reactions generate the materials for **growth**.

Role of enzymes in metabolism

In living cells, the **enzymes** regulate all the chemical reactions. **Enzyme** is a protein molecule, produced by living cells, that functions as a specific catalyst of biochemical reactions. As biocatalysts, enzymes typically **accelerate** chemical reactions.

Each enzyme acts on a specific substance called the **substrate**, and each enzyme catalyzes only one reaction.

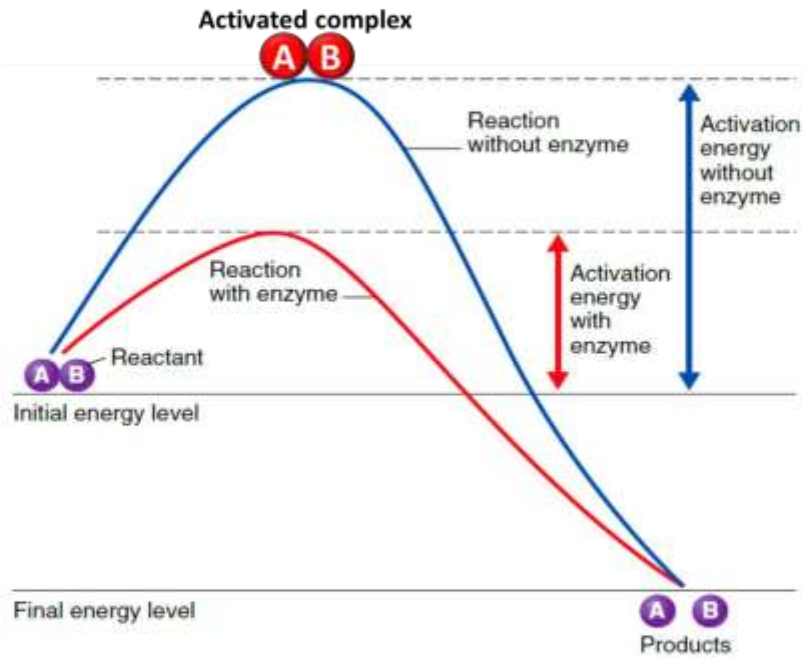
For example, sucrose (table sugar) is the substrate of the enzyme sucrase, which catalyzes the hydrolysis of sucrose to glucose and fructose (products):



How do enzymes accelerate chemical reactions?

Enzymes speed up a chemical reaction by lowering the **activation energy** required for the reactants to come together and react. Activation energy refers to the energy that is needed to trigger a chemical reaction between two or more reactants.

An enzyme's ability to accelerate a reaction without the need for an **increase in temperature** is crucial to living systems because a significant temperature increase would destroy cellular proteins. The crucial function of enzymes, therefore, is to speed up biochemical reactions at a temperature that is compatible with the normal functioning of the cell.



Production of Microbial Metabolites

1- Primary Metabolites

Primary Metabolites are involved in growth, development and reproduction. Hence, they are essential for survival and existence of the organism and reproduction.

Primary Metabolites are produced in trophophase during **Log (exponential growth) phase** in a batch culture as normal end products of primary metabolism. Macromolecules such as **proteins**, **nucleic acids** and other **cell constituents** are synthesized in primary metabolism. When primary metabolism is stopped the **organism dies**.

Table: Some industrial products resulting from primary metabolism.

Anabolic Products	Catabolic Products
1. Enzymes products, e.g. wines 2. Amino acids 3. Vitamins 4. Polysaccharides 5. Baker's Yeast 6. Nucleic acids 7. Citric acid	1. Ethanol 2. Butanol 3. Acetone 4. Lactic acid 5. Acetic acid (vinegar)

There are two types of primary metabolites:

(a) Primary essential metabolites

1- Bacteria produce such metabolites in adequate quantities.
2- These are important to sustain bacterial cell growth.
3- These compounds can be manipulated for industrial production (overproduction).
4- Examples: enzymes, vitamins, amino acids, nucleosides … etc.

(b) Primary metabolic end products

1- These are the end products of fermentation process.
2- Primary metabolic end-products are not significant function to the organism. 3- Instead, they have many industrial applications.
4- Examples: ethanol, acetone, lactic acid … etc.

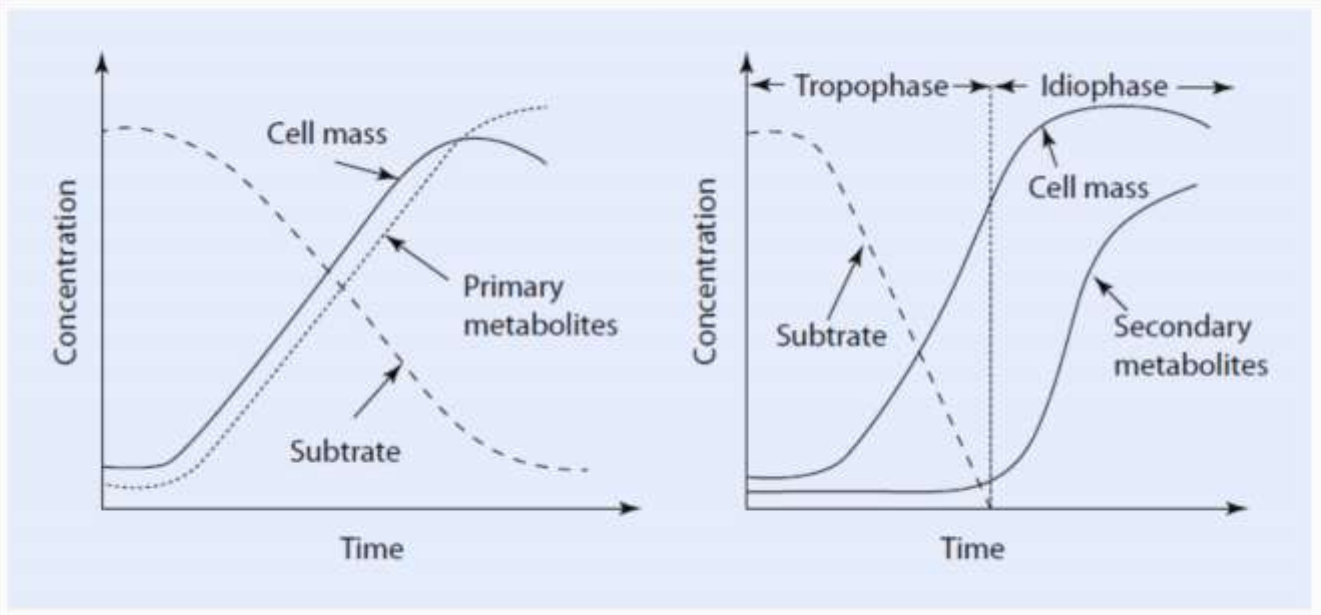


Figure. Secondary vs Primary metabolites.

2- Secondary Metabolites

Secondary metabolites are **low-molecular-weight compounds that are not required for the organism growth**. Synthesis of secondary metabolites occurs in the **late logarithmic**, and in the **stationary phase** in the growth cycle (**idiophase**).

The production of secondary metabolites starts when growth is limited with exhaustion of one key nutrient source (i.e., carbon, nitrogen, or phosphate). For example, penicillin biosynthesis by *Penicillium chrysogenum* starts when glucose is exhausted from the culture medium and the fungus starts consuming lactose, a less readily utilized glucose.

Secondary metabolites provide different functions for the microorganisms. For example:

- It protects cell from predation and environments stress
- Used for communication (cell-cell signaling).
- Used in competition and toxicity against other microbes.

Microorganisms produce a wide variety of secondary metabolites, many of which display actual or potential therapeutic application. **Antibiotics** are the most numerous such substances and this family of pharmaceuticals has greatest due to their positive impact upon human healthcare. Moreover, microorganisms produce secondary metabolites such as toxins, alkaloids, fatty acids, ketones, alcohols, etc.

Table 2 Some industrial products of microbial secondary metabolism.

Product	**Organism**	**Importance**
Antibiotics		
Penicillin	*Penicillium chrysogenum*	Clinical use
Streptomycin	*Streptomyces griseus*	Clinical use
Anti-tumor Agents		
Actinomyin	*Streptomyces antibioticus*	Clinical use
Bleomycin	*Streptomyces verticulus*	Clinical use
Toxins		
Aflatoxin	*Aspergiulus flavous*	Food toxin
Amanitine	*Amanita* sp.	Food toxin
Alkaloids		
Ergot alkaloids	*Claviceps purpurea*	Pharmaceutical
Miscellaneous		
Gibberellic acid	*Gibberalla fujikuroi*	Plant growth hormone
Patulin	*Penicillium urticae*	Anti-microbial agent

The differences between primary and secondary metabolites are:

Primary metabolites	**Secondary metabolites**
Produced during growth phase of cell, called "Trophophase"	Produced during non-growth phase, called "Idiophase" (near completion) of cell growth
Accumulation large quantities, so the downstream processing is easy.	Accumulation very small quantities so the downstream processing is complex.
Easy production process	Complex production process
Examples: Proteins, enzymes, nucleic acids, carbohydrates, amino acids, vitamins, ethanol etc.	Examples: Antibiotics, pigments, toxins etc.

Strain Improvement

As mentioned above, microorganisms can generate a wide range of valuable products, including primary and secondary metabolites. In most cases, the amount of these metabolites produced by **wild-type microorganism is too low for commercially applications**. Therefore, strain improvement is performed in order to enhance their metabolic capacities for biotechnological applications.

Strain improvement could target:

- Rapid growth
- Genetic stability
- Non-toxicity to humans
- Large cell size, for easy removal from the culture fluid (downstream processing). ▪ Ability to use cheaper substrates.
- Elimination of the production of by-products that interfere with downstream processing
- Increase productivity.

Strain improvement can be performed by different strategies:

(1) Optimizing environmental conditions, includes:

- Modification of physical parameter (temperature, agitation, etc.)
- Modification of chemical parameter (pH, O_2 concentration)
- Modification of biological parameter (enzymes)

(2) Optimizing nutrition of microorganisms, includes:

- Carbon sources
- Nitrogen sources
- Mineral sources
- Precursors

(3) Genetic improvement, includes:

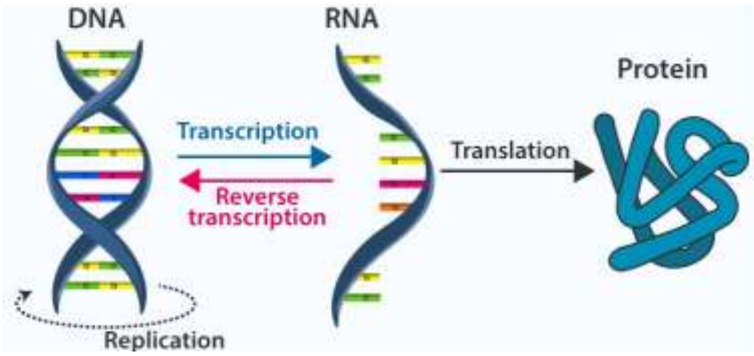
- Method not involving foreign DNA (Classical genetic methods, Mutagenesis).
- Method involving Foreign DNA (recombination DNA, Genetic engineering).

Improving wild-type strains through genetic manipulation provides a very attractive and practical approach for developing industrial microorganisms.

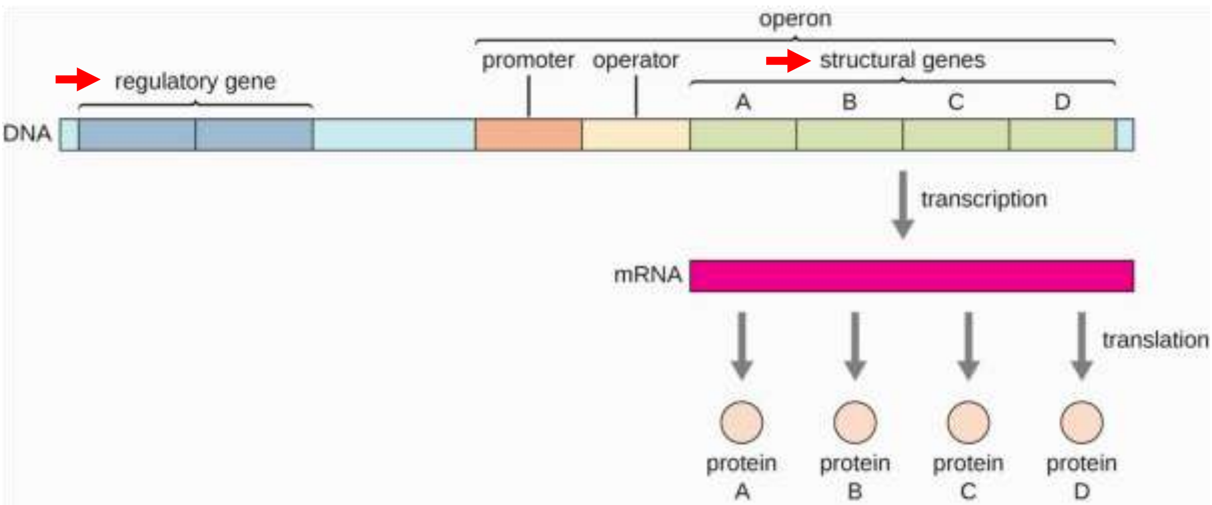
Lecture 8

Genetics and Biotechnology

All properties of organisms depend on the sum of their genes. Genes are pieces of DNA that contain genetic information. Gene is the unit of hereditary information that occupies a fixed position (locus) on a chromosome. Therefore, the precise role of DNA is to act as a reservoir of genetic information.
The flow of genetic information from DNA to RNA to protein (known as **central dogma**). It is the process by which the information in DNA is converted into a functional product (proteins). Hence, the information present in a DNA is essential to make up all proteins. RNA acts as a messenger that carries information through the ribosomes to create proteins via translation.



There are two types of genes: **structural genes** and **regulatory genes**



Structural genes encode for amino acid sequences of proteins, which, acting as enzymes that speed up metabolism or chemical reactions in cells or, alternatively, act as components of cellular structures such cell wall.

Regulatory genes regulate the expression of the structural genes by controlling the rate of production of proteins in response to **intra- or extracellular signals**.

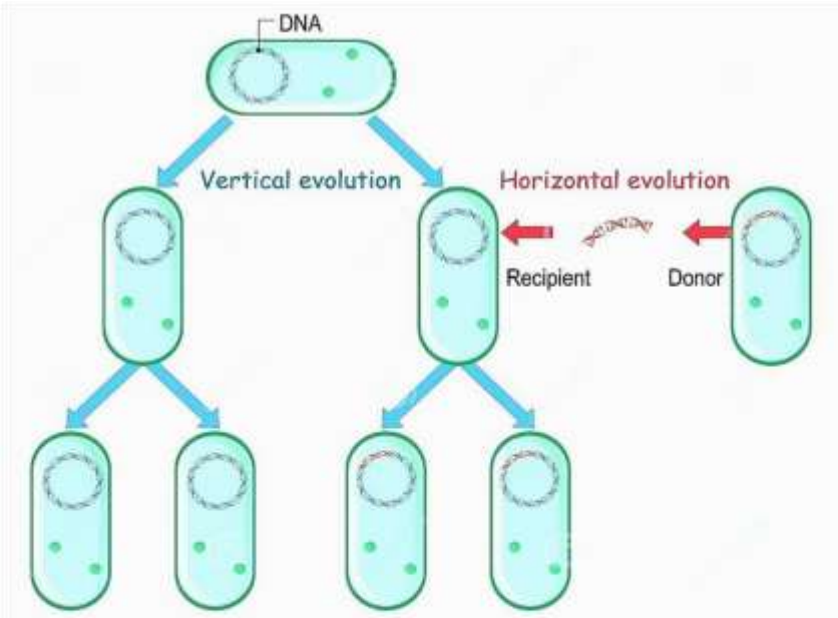
Humans have been manipulating living things for thousands of years. Examples of early biotechnologies include domesticating plants and animals and then **selectively breeding them for specific characteristics**.

The discovery of the structure of DNA by the studies of Watson and Crick and others in the early 1950s led to new research in areas such as gene therapy for genetic disease, the sequencing of the human genome and modern biotechnology that based on our understanding of gene structure and function.

Changes in the DNA molecule are the means by which organisms develop and adapt themselves to new environments. **In nature, these changes in the DNA of an organism can occur in two ways:**

(1) **by genetic mutation**, which is a change in the structure of a gene; deletion or addition of one or more nucleotides.

(2) **by the interchange of genetic information** (DNA) between similar organisms normally by sexual reproduction (vertical, from parents to offspring), and by horizontal gene transfer in bacteria such as the spread of antibiotic resistance genes among bacteria:



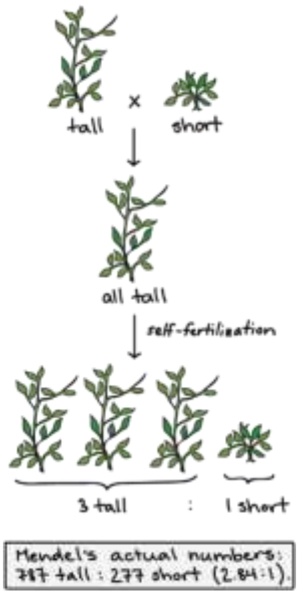
Genetics: from Classical to Modern Biotechnology

Humans have been manipulating living things for thousands of years. Examples of early biotechnologies include domesticating plants and animals and then selectively breeding them for specific characteristics.

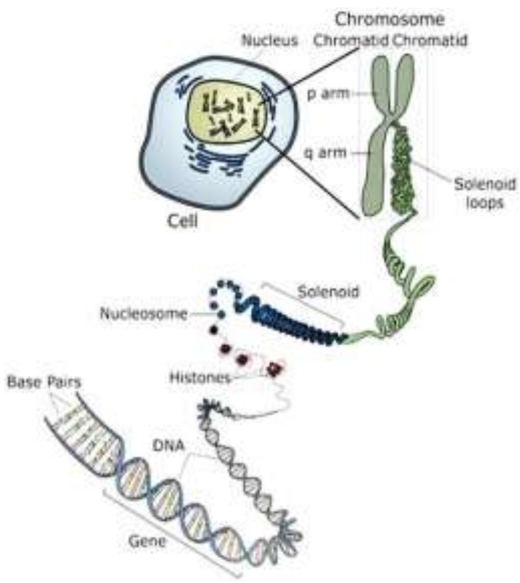
Genetics is the study of heredity in general and of genes in particular. Genetics forms one of the central pillars of biotechnology. As we mentioned in the previous lectures, biotechnology is the use of living systems and organisms to develop or make useful products. Genetic developments are allowing to make new biotechnological products such as new medicines, new foods, solve the environmental problems …etc.

Classical Biotechnology

Classical Biotechnology is the second stage in development of biotechnology. This stage existed from 1800 to almost the middle of the twentieth century. During this period various observations started pouring in, with scientific evidences.



The basics for the transfer of genetic information are the core of biotechnology. This was, for the first time, applied in plants such as Pea plant (*Pisum sativum*). These observations were explained by Gregor John Mendel (1822-1884) presented as "**Laws of Inheritance**" to the Natural Science Society in Brunn, Austria.



Almost at the same time Robert Brown had discovered nucleus in cells, while in 1868, Fredrich Miescher, a Swiss biologist isolated **nuclein** (DNA associated with proteins) from cell nuclei that he extracted from human pus cells. These two discoveries became the basis of **modern molecular biology**, for the discovery of DNA as a genetic material, and the role of DNA in transfer of genetic information.

In 1888, Heinrich Wilhelm Gottfried Von Waldeyer-Hartz, a German scientist coined the term "**Chromosome**", which is considered as an organized structure of coiled DNA and protein present in cells containing many genes.

In 1909, the term "**Gene**" was coined by Wilhelm Johannsen (1857-1927), who described **gene** as carrier of heredity.

In 1926, the principle of genetics in inheritance was redefined by Morgan, who has shown inheritance and the role of chromosomes in inheritance by using fruit flies

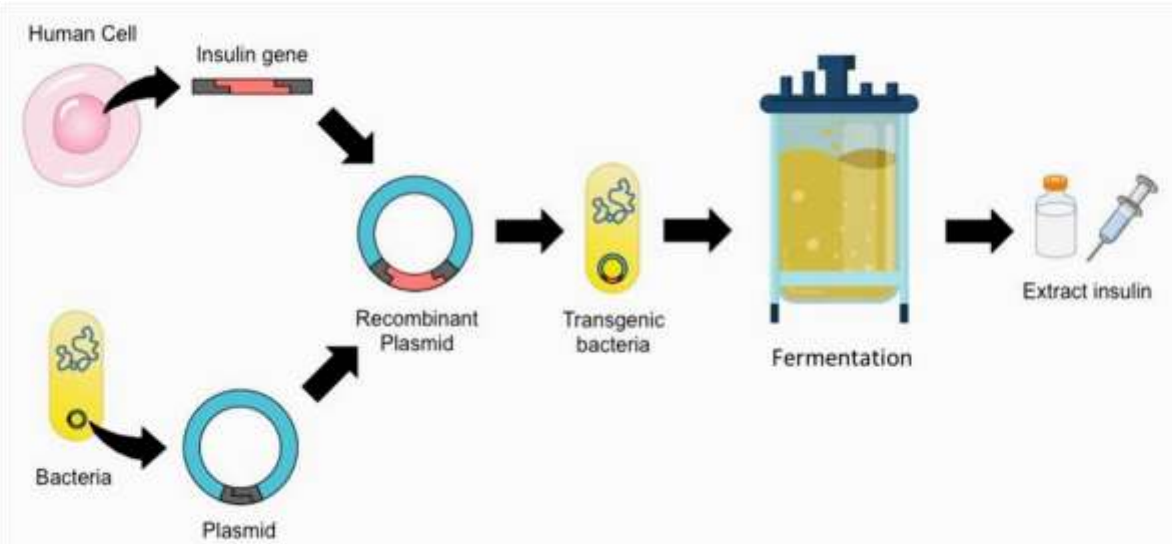
(*Drosophila melanogaster*). This landmark work of Morgan was named "**The theory of the Gene**".

Johannsen coined the terms "**genotype"** describe the entire genetic constitution of an individual, and "**phenotype"** which describe the observable properties of an organism that influences by the genetic constitution of the organism and the effects of environmental factors.

Modern Biotechnology

Since the discoveries of Mendel in heredity, it took another 90 years of research before the structure of DNA was described in **1953 by Watson and Crick**. **This discovery was the beginning of modern biotechnology**. Modern biotechnology is based on the understanding of DNA structure, which allows scientists to develop and produce new desired products, such as insulin.

Modern biotechnology originated in 1973 with the invention of recombinant DNA technology that dramatic changes in biotechnology by allowing scientists to cut and join different pieces of DNA, and place the new recombinant DNA (Chimeric/hybrid DNA) into a new host. This technique allows the transfer of gene(s) from one organism to another conferring a novel property. Since the emerge of rDNA technology, biotechnology has developed and led to advancements in medicine, agriculture and environmental science. The figure below shows the genetically modified bacteria (*E. coli*) producing recombinant human insulin which marketed in the United States in 1982.



After the end of the Second World War some, very crucial discoveries were reported, which paved the path for modern biotechnology and to its current status:

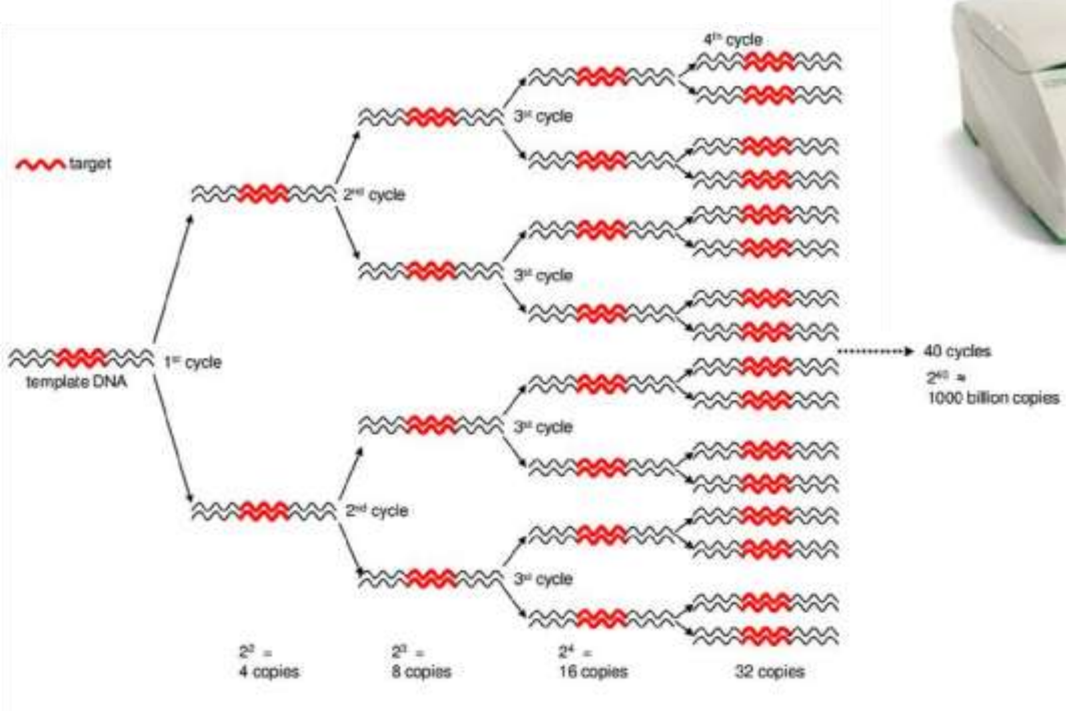
In 1953, Watson and Crick for the first time cleared the mysteries around the DNA as a genetic material, by giving a structural model of DNA, popularly known as, "**Double**

Helix Model of DNA”. This model was able to explain various phenomena related to DNA replication, and its role in inheritance.

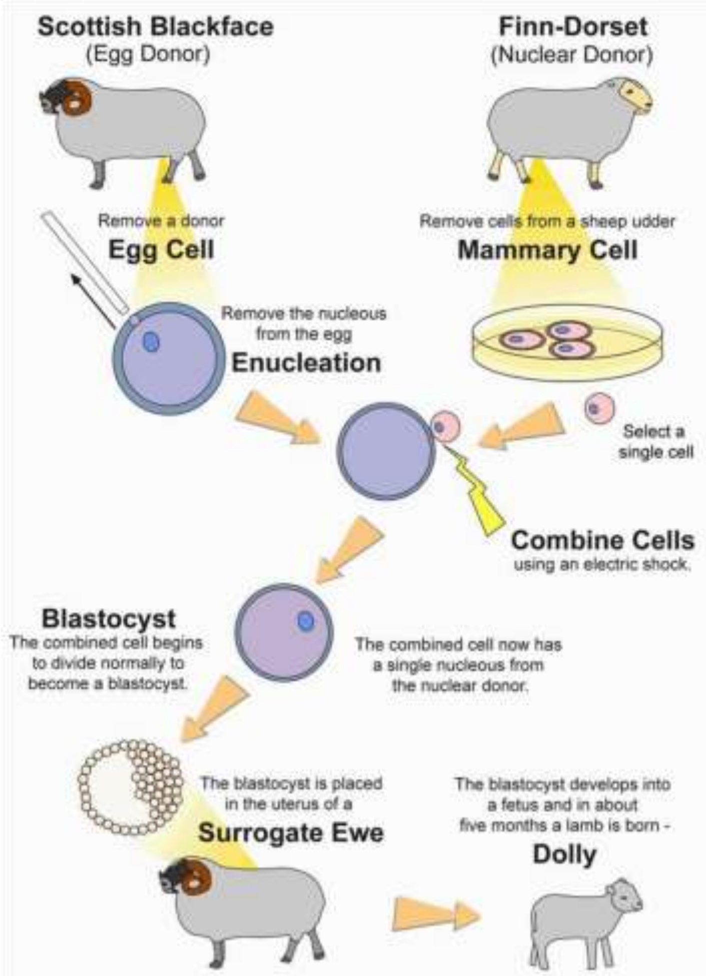
Dr. Hargobind Khorana was able to synthesize the DNA in test tube. In 1970, **Khorana** published the results of his research on creating a **synthetic gene, a piece of DNA that he had made in a test tube**. This was the first time anyone made a DNA molecule long enough to contain all the information needed to make a gene for a transfer RNA (tRNA).

In 1970, **Restriction enzymes** are discovered. These enzymes cut DNA at specific sites into pieces and are used for various studies and applications. The restriction enzyme technique becomes a fundamental tool in modern genetic research and opens the way for gene cloning.

In 1993, Kary Mullis an American biochemist, received the Nobel Prize for Chemistry for his invention of the **Polymerase Chain Reaction (PCR)**, a simple laboratory technique used to make millions or billions of a particular region of DNA in short time. This DNA region can be a gene a researcher wants to understand. This technique allows the scientists to **insert a foreign DNA into another host** and to **monitor** the transfer of a foreign DNA into the next generation.



sheep as model, and he named the cloned sheep as “**Dolly**”.



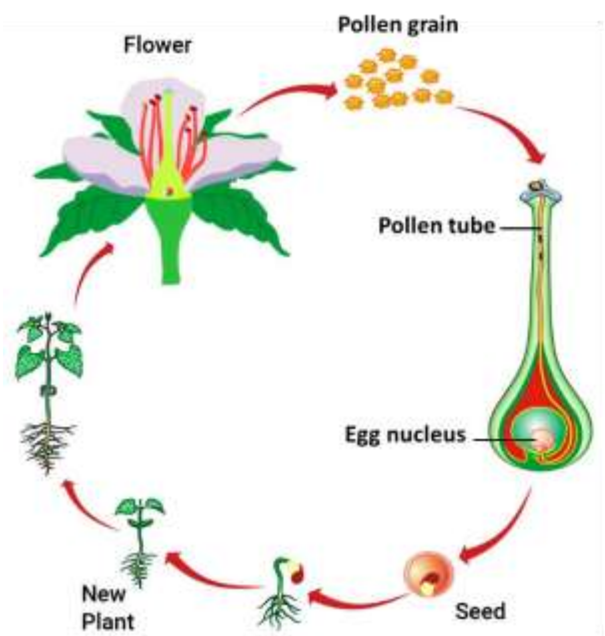
In 1996, **Ian Wilmut** an Irish scientist was successful to clone an adult animal, using
The Human Genome Project (HGP) was operated from 1990 to 2003 provides researchers with basic information about the sequences of the three billion nitrogenous base (i.e., adenine [A], thymine [T], guanine [G], and cytosine [C]) that make up human genomic DNA.
Human Genome Project has opened up a new era in modern biotechnology that may improve life quality of human with unlimited applications. In medicine, for example, help to understand diseases that have a genetic basis, such as cancer and diabetes, improve diagnosis of diseases and design new medicines.

Genetic Manipulation

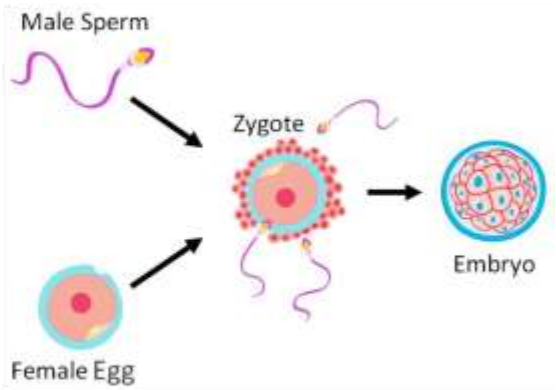
The manipulation of the genetic material in organisms can be achieved in three ways: **organismal**, **cellular** and **molecular** manipulation.

1) Organismal manipulation

Genetic manipulation of **whole organisms** is happening naturally by **sexual reproduction** since the beginning of time. Organismal manipulation was practised in agriculture for decades, even centuries. It is also used with several industrial microorganisms, e.g. brewing yeasts. This method involves selection, mutation, sexual crosses, etc. In agriculture, the benefits are enormous with much improved plants and animals. In industrial biotechnology, there have been greatly improved productivities, e.g. antibiotics and enzymes.



Sexual Reproduction in Plants

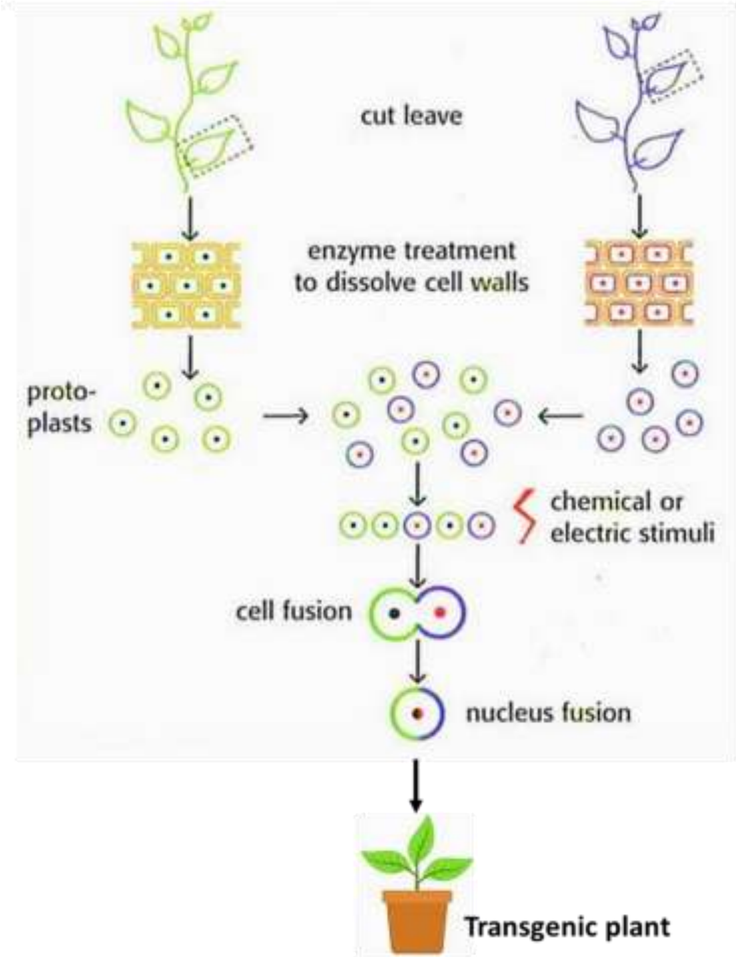


Sexual Reproduction in Animals

2) Cellular manipulation

Cellular manipulations of DNA involve either **cell fusion** and the regeneration of whole plants from these cells with desirable traits, like quality and yield. Cell fusion is a genetic engineering process in which the nucleus is removed from a plant cell and replaced by a nucleus from a different plant that **might be from a different type** by means of methods that do not occur naturally. This creates a new plant with **mixed**

genetics. This fundamental biological process has been well documented in many organisms, including plants, yeast, and higher eukaryotes.



3) Molecular manipulation

Molecular manipulations of DNA and RNA (knowns as genetic engineering or recombinant DNA technology) enabling **a directed control of the changes**. These manipulations are bringing dramatic changes to biotechnology. With this technology, it now possible to add or delete parts of the DNA molecule with high precision and the products can be easily identified. Current industrial trends are concerned with the production of new genetically engineered organisms and of numerous new compounds such as pharmaceuticals.

Principles of Biotechnology II

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Level 1
Second Semester
2024 - 2025

Lecture 9

Molecular Manipulation of Genetic Material
Genetic Engineering

Molecular Biotechnology

As we mentioned in the previous lecture (Lecture 4), modern biotechnology has established after the discovery of genetic material and the central dogma of the genetic information flows, namely DNA → RNA → Proteins, during the 1930s to the 1950s. These discoveries lead to dramatic changes in biotechnology through modification of genetic material of organisms and establish a new branch called **Molecular biotechnology**.

Molecular biotechnology is the use of laboratory techniques to study and modify nucleic acids and proteins for applications in areas such as human and animal health, agriculture, and the environment.

Molecular biotechnology can contribute benefits to humanity:

1- Provide opportunities to accurately diagnose, prevent, or cure a wide range of infectious and genetic diseases.
2- Significantly increase crop yields by creating plants that are resistant to insect pests, microbial diseases, and environmental stresses, such as drought, and at the same time reduce using of hazardous agrichemicals.
3- Develop microorganisms produces chemicals, antibiotics, enzymes, and food additives that are important for food production and other industries.
4- Improving economically important traits in animals such as quality of meat, milk composition, disease resistance.
5- Facilitate the removal of pollutants and waste materials from the environment using genetically modified microbes.

Although it is exciting and important benefits of molecular biotechnology, there are also social concerns and consequences that must be addressed such as:

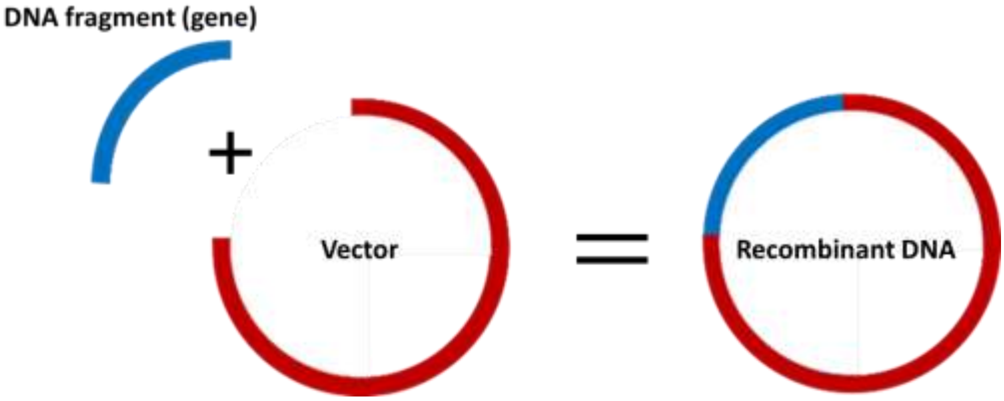
- Will some genetically engineered organisms be harmful either to other organisms or to the environment?
- Should humans be genetically engineered?
- Will new diagnostic procedures undermine individual privacy?
- Will agricultural molecular biotechnology undermine traditional farming practices?

Genetic Engineering/ Recombinant DNA Technology

Recombinant DNA Technology

Scientists can modify the DNA of bacteria, plants and animals to add genetic information from a different organism. The process is horizontal gene transfer from bacteria to vertebrates. This process has historically been called genetic engineering but more recently is referred to as recombinant DNA (rDNA) technology or genetic modification. So, in molecular biotechnology, recombinant DNA technology, gene cloning, and genetic engineering, in a broad sense, have the same meaning.

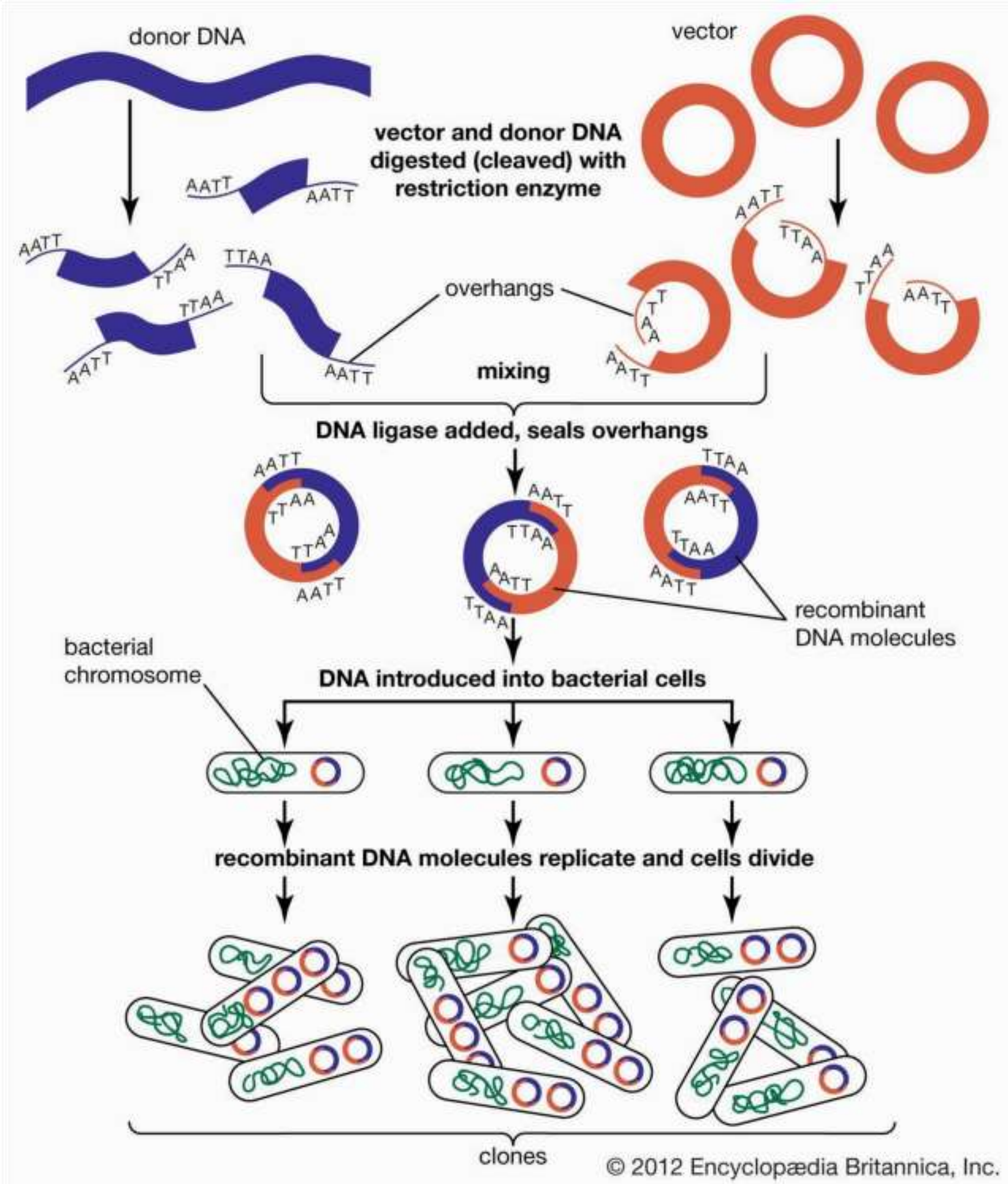
Recombinant DNA technology are a techniques used for production of recombinant DNA *in vitro* and transfer of the recombinant DNA into cells where it may be expressed or propagated.



rDNA technology is a major arm of genetic engineering which has been applied to the manufacturing of pharmaceuticals, particularly therapeutic proteins such as insulin, human serum albumin, human papillomavirus vaccine, and hepatitis B vaccine.

Basically, rDNA technology essentially involves isolating a gene of interest, inserting the gene into a cloning vector, and allowing the gene product to be expressed within an appropriate host as shown in the figure below:

Figure below shows the process of rDNA:



A: Adenine
T: Thymine

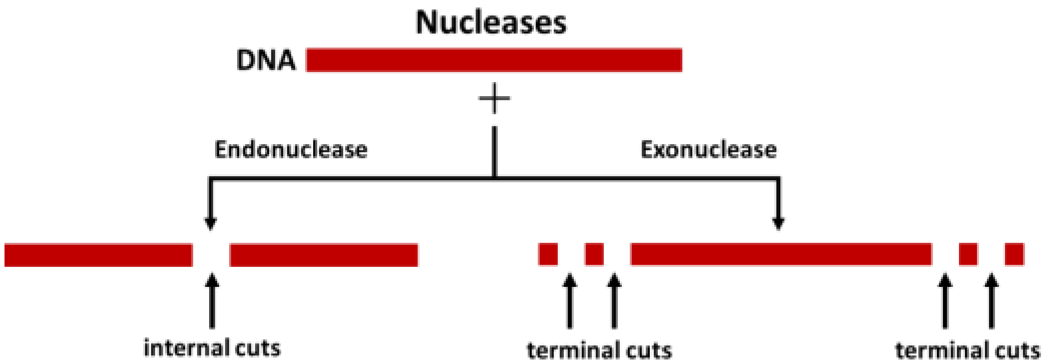
Tools of rDNA Technology

(1) Restriction Enzymes (also known as Restriction Endonucleases)

Restriction enzymes (nucleases) are enzymes that **recognize specific double stranded DNA sequences** and cleave the DNA in both strands at these sequences. There are two types of Nucleases:

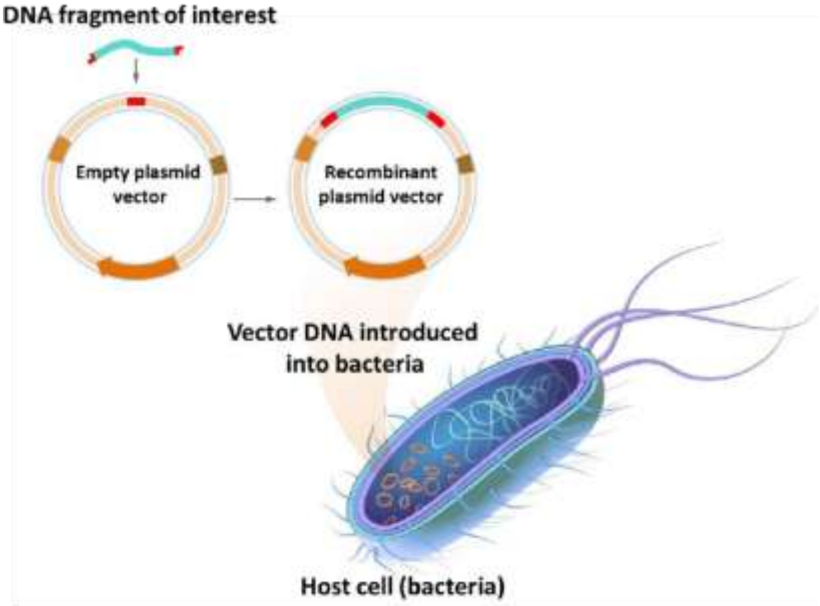
Endonucleases that are cut nucleic acid molecules internally

Exonucleases that are degrade from the ends of nucleic acids are.



(2) Vectors

A vector is a DNA molecule that is used as a vehicle to carry a particular DNA segment into a host cell. There are four major types of vectors are plasmids, viral vectors, cosmids, and artificial chromosomes.



(3) Host

The DNA insert cloned in the vector should be transformed inside a host for multiplication. Bacteria such as *E. coli* is the most frequently used host for production of enzymes and other proteins by recombinant DNA technology because its simplicity, inexpensive and fast high-density cultivation, well-known genetics, and large number of compatible molecular tools available.

Steps in rDNA Technology (see the Figure on page 33)

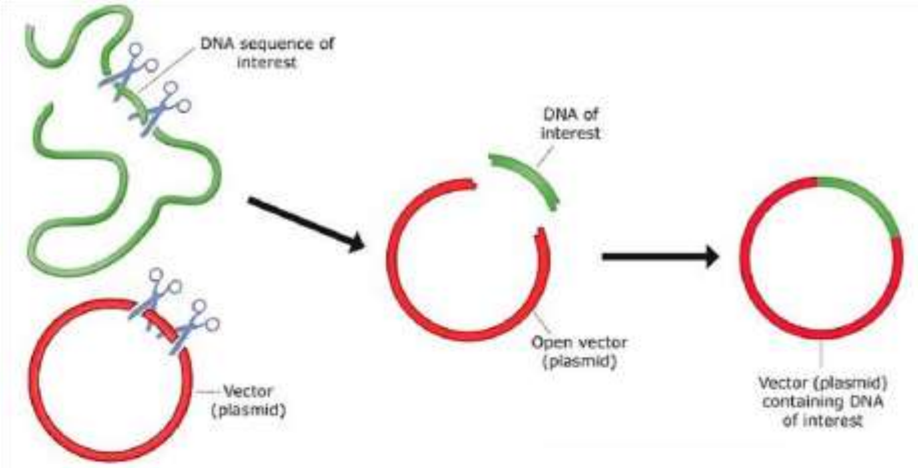
I. Isolation of the Desired Gene

In order to achieve the rDNA steps, cut the DNA with restriction enzymes, DNA should be in pure form, free from other macro-molecules and impurities. Since the DNA is enclosed within the membranes, we have to break the cell open to release DNA along with other macromolecules such as RNA, proteins, polysaccharides and also lipids. This can be achieved by physical, chemical and enzymatic methods. Other molecules can be removed by appropriate treatments and purified DNA ultimately precipitates out after the addition of chilled ethanol. ***Please read the Lab lecture experiments (Lab. 3 and Lab. 4).***

2. Inserting the desired DNA fragment into a vector

Vectors are small, independently replicating genetic elements used to carry and replicate cloned DNA segments. **Most vectors are plasmids or viruses**. Vectors are typically designed to allow insertion of foreign DNA at a restriction site that cuts the vector without affecting its replication. The steps of vector creation are:

1- Cutting out the gene
2- Opening up the vector
3- Sticking the vector and the gene together



3. Introduction of the cloned DNA into a host organism

Recombinant DNA molecules made *in vitro* are introduced into suitable **host organisms** where they can replicate. **Transformation** is often used to insert recombinant DNA into cells.

Lecture 10

Applications of Genetic Engineering in Biotechnology

Human life is greatly affected by three factors: **deficiency of food**, **health problems**, and **environmental issues**. Food and health are basic human requirements beside a clean and safe environment.

- With increasing world's population at a greater rate, human requirements for food are rapidly increasing. Humans require safe-food at reasonable price.
- Several human related health issues across the globe cause large number of deaths. Approximately 36 million people die each year from noncommunicable and communicable diseases, such as cardiovascular diseases, cancer, diabetes, AIDS, tuberculosis, malaria, and several others.
- Despite extensive efforts, the current world food production is much lower than human requirements, and health facilities are even below standard in the thirdworld countries.
- Rapid increase in human activities in industry and agriculture generate industrial wastes, domestic sewage and toxic waste cause water and soil pollution by contaminating water with infectious microorganisms and toxic substances.

Therefore, these issues urge to be addressed through modern biotechnological methods such as applying genetic engineering/rDNA techniques.

Genetic Engineering in Agriculture

Genetically modified (GM) plants have been useful in many ways. Genetic modification has:

1- Made crops more tolerant to abiotic stresses (cold, drought, salt, heat).
2- Reduced reliance on chemical pesticides (pest-resistant crops).
3- Helped to reduce post-harvest losses.
4- Increased efficiency of mineral usage by plants (this prevents early exhaustion of fertility of soil).
5- Enhanced nutritional value of food, e.g., vitamin A enriched rice.
6- GM used to create plants for specific uses in industry in the form of starches, 7- Fuels and pharmaceuticals.

Production of Pest Resistant Plants

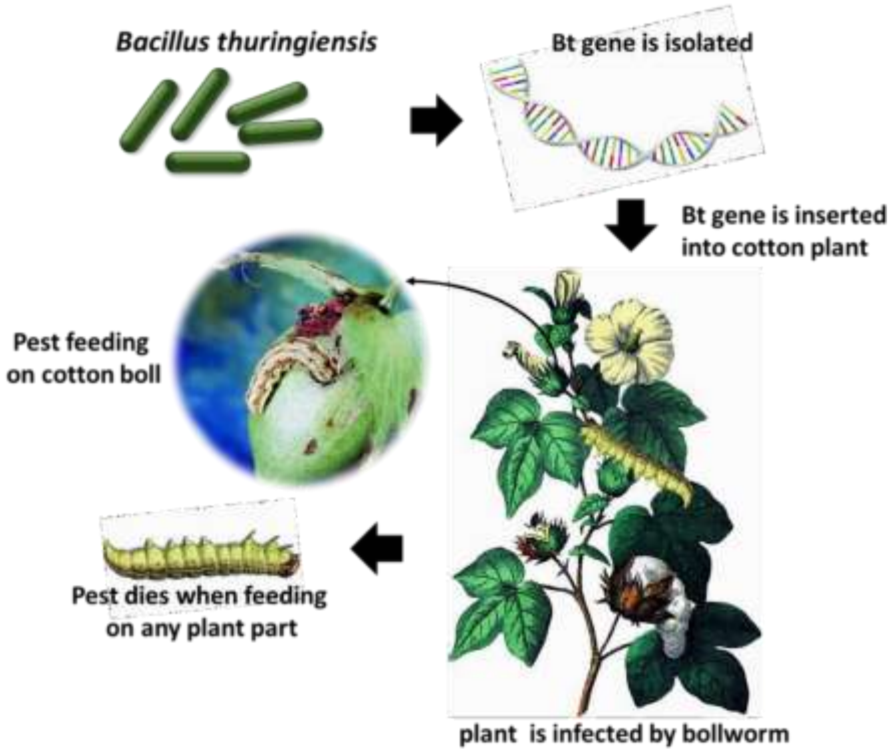
Pest resistant plants could decrease the amount of pesticide used. Bt toxin is produced by a bacterium called ***B**acillus **t**huringiensis*. Bt toxin gene has been cloned from the bacteria and been expressed in plants to provide resistance to insects without the need for agrochemical pesticides. Examples are Bt cotton, Bt corn, rice, tomato, and potato… etc.

Bt Cotton

Bt cotton is one of the first crop protection products from biotechnology. Some strains of *Bacillus thuringiensis* produce proteins that kill certain insects such as lepidopterans (tobacco budworm, armyworm), coleopterans (beetles) and dipterans (flies, mosquitoes). *B. thuringiensis* forms protein crystals during a particular phase of their growth. These crystals contain a toxic insecticidal protein.

Why does this toxin not kill the *Bacillus*? Actually, the Bt toxin protein exist as inactive protoxins but once an insect ingests the inactive toxin, it is converted into an active form of toxin due to the alkaline pH of the gut which solubilise the crystals. The activated toxin binds to the surface of midgut epithelial cells and create pores that cause cell swelling and lysis and eventually cause death of the insect.

Specific Bt toxin genes were isolated from *Bacillus thuringiensis* and incorporated into the cotton plant, then the toxin is coded by a gene named cry (see Figure below).



Golden Rice

The World Health Organization (WHO) estimates that vitamin A deficiency affects 230 million children worldwide, and at least one million children per year are dying of diseases related to this deficiency.

Golden Rice is the name of a rice that has been genetically engineered (genetically modified or GM) to produce beta-carotene, which the body can convert into vitamin A. This beta-carotene gives the rice grains the yellowish colour that inspired its name.

Genetic Engineering in Medicine

Gene Therapy

Gene therapy is a technique that modifies or manipulate a human's genes to treat or cure disease such as cancer, genetic diseases, and infectious diseases.

Gene therapies can work by several mechanisms:

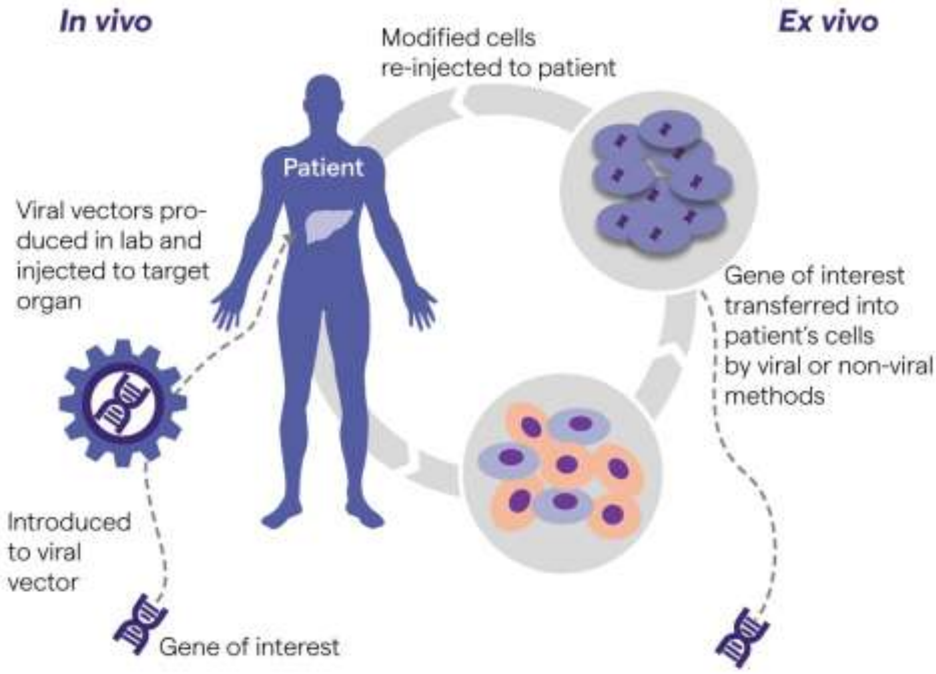
1- Replacing a disease-causing gene with a normal gene.
2- Inactivating a disease-causing gene that is not functioning properly.
3- Introducing a new or modified gene into the body to help treat a disease.

Strategies of Gene Therapy

Basically, two strategies are used in gene therapy: ***in vivo*** **gene therapy** and ***ex vivo*** **therapy**.

In vivo gene therapy: a therapeutic gene is transferred into the patient using a virus. This approach has achieved success in the treatment of neurological disorders and hemophilia.

In ex vivo therapy: a gene modification is introduced into cells isolated from a patient and then re-injected to the patient, particularly cancer.



Gene Therapy Delivery systems

Gene delivery systems include viral vectors and non-viral vectors:

Viral vectors

In vivo and *ex vivo* gene therapy using viruses utilize their inherent ability to invade cells to deliver manipulated gene. The use of viruses to transfer genetic material, known as **transduction**. This type of viral vector-based therapies used to treat diseases such as Hematologic diseases, Cardiovascular diseases, and several cancers.

Non-viral vectors

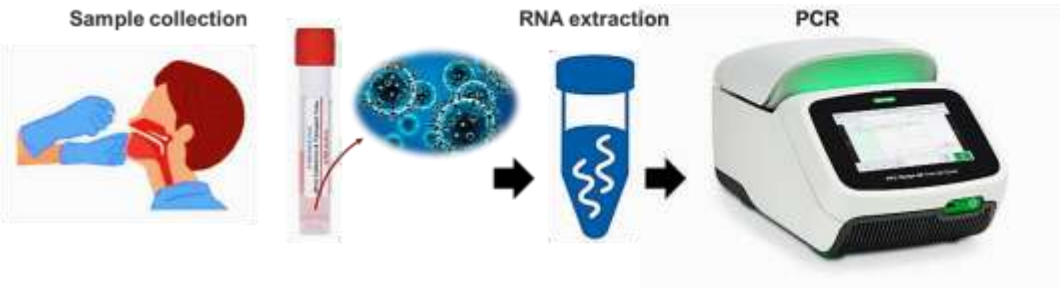
Non-viral gene therapy holds great premises as it is assumed to be less toxic for the host and much safer in terms of gene delivery compared to viral vectors. The non-viral vectors are administered by direct delivery such as plasmid DNA/Naked DNA, chemical vectors like liposomes, physical methods like electroporation and needle.

Molecular Diagnosis

For effective treatment of a disease, early diagnosis and understanding its pathophysiology is very important. Using conventional methods of diagnosis (serum and urine analysis, etc.) early detection is not possible. Recombinant DNA technology, Polymerase Chain Reaction (PCR) and Enzyme Linked Immuno-sorbent Assay (ELISA) are some of the techniques that serve the purpose of early diagnosis.

Presence of a pathogen (bacteria, viruses, etc.) is normally suspected only when the pathogen has produced a disease symptom. By this time the concentration of pathogen is already very high in the body. However, very low concentration of a bacteria or virus (at a time when the symptoms of the disease are not yet visible) can be detected by amplification of their nucleic acid by PCR.

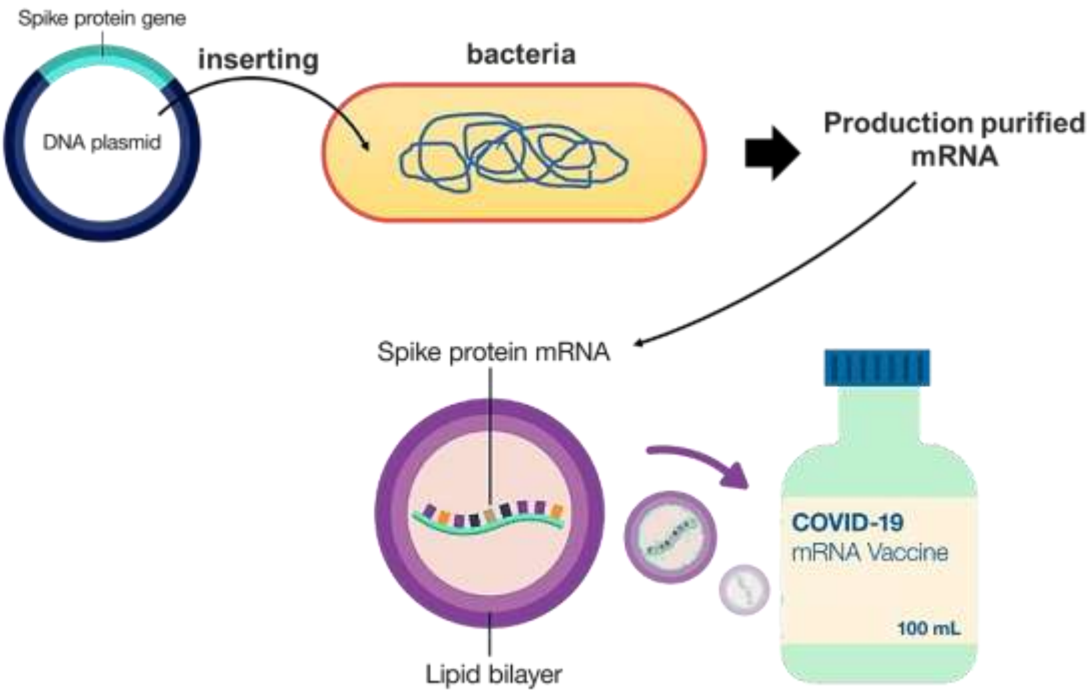
PCR is now routinely used to detect HIV in suspected AIDS patients and recently COVID-19. It is being used to detect mutations in genes in suspected cancer patients too. It is a powerful technique to identify many other genetic disorders.



Development of Vaccines

Vaccines prevent many millions of illnesses and save numerous lives every year. As a result of widespread vaccine use, some viral childhood diseases are reduced around the world or completely eradicated such as measles and polio. Recently, the scientists developed and commercialized a new mRNA vaccine against COVID-19.

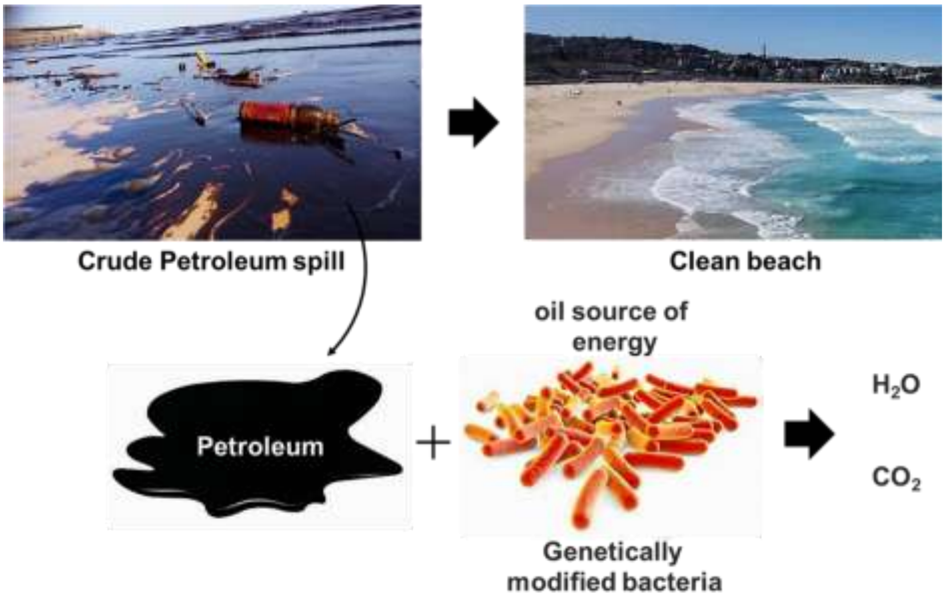
The new generation vaccine of COVID-19, mRNA encodes the genetic information to produce a target antigen, the **spike protein** that is found on the surface of the virus. This mRNA is then encapsulated in lipid nanoparticles for injection. Once injected into a person, the mRNA is taken up into the cells and their own intracellular translation machinery is triggered to produce the antigen protein. These produced antigens then trigger the body's immune response and the production of antibodies, which ultimately protect the body against future infections from that virus.



Genetic Engineering in Environment (oil-eating microbes)

Genetic engineering has wide applications in solving the environmental issues. The genetically engineered bacteria are used as active agents in petroleum degradation, and they work as primary degraders of spilled oil in environment.

The mechanism of petroleum biodegrading could be (1) either they use organic carbon deriving from hydrocarbons as alternative carbon and energy sources (2) or they accumulate them inside by as a defence mechanism.



If rDNA bacteria can clean up an oil spill but will alter the natural ecosystem, should we use them?

Ethical Issues in Modern Biotechnology

The ethical issue in biotechnology can be divided into:

- Socio-economic issues
- Cultural issues
- Legal issues
- Environmental issues
- Religious issues

Here are some ethical, legal, and social issues:

- Who owns genetically modified organisms such as bacteria? Can such organisms be patented like inventions?
- Are genetically modified foods safe to eat? Might they have unknown harmful effects on the people who consume them?

- Are genetically engineered crops safe for the environment? Might they harm other organisms or even entire ecosystems?
- Who controls a person's genetic information? What safeguards ensure that the information is kept private?
- How far should we go to ensure that children are free of mutations? Should a pregnancy be ended if the fetus has a mutation for a serious genetic disorder?