B.Tech Degree EXAMINATION

JANUARY 2023

Fifth Semester

Biomedical Engineering

ELEMENTS OF BIOTECHNOLOGY

(From 2009 -10 onwards)

Keywords of Answer

PART A

1. What are three main Classification of Micro-organisms ?(Internal Test 1 Part B QNo.12)

Monera – Unicellular prokaryotes. Protista – Unicellular eukaryotes. Fungi – Eukaryotic, heterotrophic (saprophytic/ parasitic) and with a cell wall (chitin).

2. Write the significance of RNA? (Internal Test 1 Part A QNo 3, Part B QNO 13)

rRNA: It helps bind the mRNA to the ribosomal surface and form peptide bonds between amino acids during protein synthesis.

mRNA: It conveys the genetic information in the form of codons from the DNA to the ribosomes.

tRNA: It acts as an adaptor for attaching amino acids to the mRNA template during protein synthesis.

3. List any four applications of enzymes? (Internal Test 1 Part A QNo 7)

They are used in foods and beverages processing, animal nutrition, textiles, household cleaning and fuel for cars and energy generation..

4. Explain about of the formation of peptide bond with an Example? (Model Exam Part A QNO. 3)

A peptide bond, also referred to as an amide bond, is formed between the α-nitrogen atom of one amino acid and the carbonyl carbon of a second (diagrammed below). So-called

isopeptide bonds refer to amide bonds between sidechain amines or carbonyl carbons on the side chain rather than α-amine or α-carbonyl.

5. Write brief notes on gene therapy and its type? (Internal test 1 Part A QNo 10. Model exam Part A QNo. 6)

Gene therapy is a technique that modifies a person's genes to treat or cure disease. Gene therapies can work by several mechanisms:

- Replacing a disease-causing gene with a healthy copy of the gene
- Inactivating a disease-causing gene that is not functioning properly
- Introducing a new or modified gene into the body to help treat a disease

There are a variety of types of gene therapy products

- Plasmid DNA
- Viral vectors
- Bacterial vectors
- Human gene editing technology
- Patient-derived cellular gene therapy products

6. Differentiate insitu and exsitu bioremediation with a suitable example?

In situ bioremediation process is performed at the original site of the contaminant.

Ex situ bioremediation process is performed out of the location where the contaminant is found.

7. Name any two restriction enzymes and add note on its site of splicing.(Internal1 Part B QNo.14)

- Type I. These restriction enzymes cut the DNA far from the recognition sequences. ...
- Type II. These enzymes cut at specific positions closer to or within the restriction sites. ...
- Type III. These are multi-functional proteins with two subunits- Res and Mod. ...
- In Gene Cloning.

8. Write the principle behind pairwise sequence alignment?

In bioinformatics, a sequence alignment is a way of arranging the sequences of DNA, RNA, or protein to identify regions of similarity that may be a consequence of functional, structural, or evolutionary relationships between the sequences.

The principle behind pairwise sequence alignment

- Sequence alignments are the starting points for methods predicting de novo the secondary structure of proteins,
- They are a preerequisite for all knowledge-based tertiary structure predictions,

- For the estimation of the total number of different types of protein folds,
- For interpreting data of genome sequencing projects and
- For inferring phylogenetic trees and resolving questions of ancestry between species.

9. How PCR is used for plant diagnostics?

PCR-based techniques use the total DNA from the diseased plant tissue as a template to detect the target organisms.

PCR is commonly used in plant genetics and molecular breeding to copy a specific DNA fragment from the genome of an individual as a step in the process of molecular marker assisted selection.

10. List any four microorganisms used in food and beverage Industry

Microbes such as bacteria, molds, and yeasts are employed for the foods production and food ingredients such as production of wine, beer, bakery, and dairy products.

PART B

UNIT 1

11. Write an essay an Cellular metabolism. (Internal Test 1 Part B QNO 12) (11 Mark)

Cellular metabolism involves a series of interconnected biochemical reactions catalysed by enzymes inside a cell, better known as metabolic pathways. These reactions convert a substrate molecule or molecules through a series of metabolic intermediates, eventually yielding a final product. These processes allow organisms to grow and reproduce, maintain their structures, and respond to environmental changes.

Amsbio offers an extensive range of proteins, antibodies, assays and kits covering every single target of major cellular metabolic pathways including folate metabolism, pyruvate metabolism (with and without oxygen), citrate metabolism, O2 consumption and toxicity, oxidative stress and fatty acid oxygen measurement.

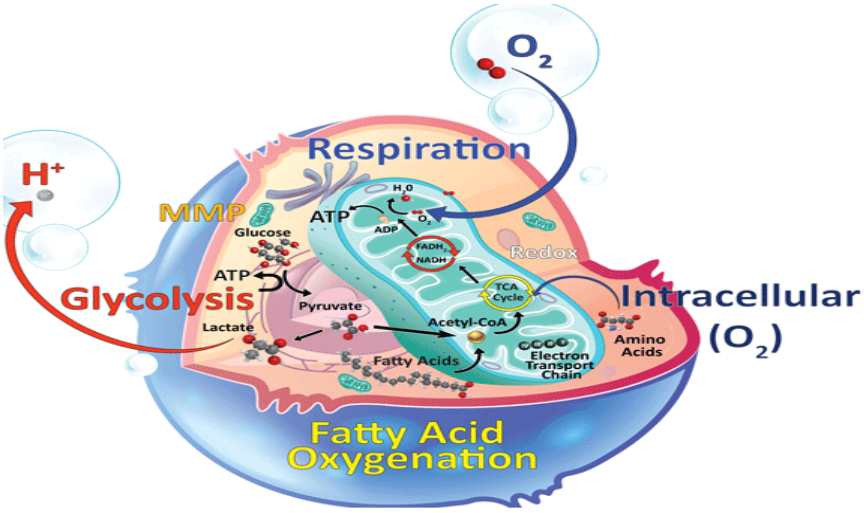


Figure Cellular metabolism.

- The totality of an organism's chemical reactions is called metabolism.
- Metabolism is an emergent property of life that arises from interactions between molecules within the orderly environment of the cell.

The chemistry of life is organized into metabolic pathways.

- Metabolic pathways begin with a specific molecule, which is then altered in a series of defined steps to form a specific product.
- A specific enzyme catalyzes each step of the pathway.
- Catabolic pathways release energy by breaking down complex molecules to simpler compounds.
    - A major pathway of catabolism is cellular respiration, in which the sugar glucose is broken down in the presence of oxygen to carbon dioxide and water.
- Anabolic pathways consume energy to build complicated molecules from simpler compounds. They are also called biosynthetic pathways.
    - The synthesis of protein from amino acids is an example of anabolism.
- The energy released by catabolic pathways can be stored and then used to drive anabolic pathways.
- Energy is fundamental to all metabolic processes, and therefore an understanding of energy is key to understanding how the living cell works.
    - Bioenergetics is the study of how organisms manage their energy resources.

Organisms transform energy.

- Energy is the capacity to do work.
    - Energy exists in various forms, and cells transform energy from one type into another.
- Kinetic energy is the energy associated with the relative motion of objects.
    - Objects in motion can perform work by imparting motion to other matter.
    - Photons of light can be captured and their energy harnessed to power photosynthesis in green plants.

    - Heat or thermal energy is kinetic energy associated with the random movement of atoms or molecules.
- Potential energy is the energy that matter possesses because of its location or structure.
    - Chemical energy is a form of potential energy stored in molecules because of the arrangement of their atoms.
- Energy can be converted from one form to another.
    - For example, as a boy climbs stairs to a diving platform, he is releasing chemical energy stored in his cells from the food he ate for lunch.
    - The kinetic energy of his muscle movement is converted into potential energy as he climbs higher.
    - As he dives, the potential energy is converted back to kinetic energy.
    - Kinetic energy is transferred to the water as he enters it.
    - Some energy is converted to heat due to friction.

(or)

12. Elaborate the Cell structure and functions with neat diagram (Internal Test 1 Part B QNo. 11).(11 Mark)

Cell Definition

"A cell is defined as the smallest, basic unit of life that is responsible for all of life's processes."

Cells are the structural, functional, and biological units of all living beings. A cell can replicate itself independently. Hence, they are known as the building blocks of life.

Each cell contains a fluid called the cytoplasm, which is enclosed by a membrane. Also present in the cytoplasm are several biomolecules like proteins, nucleic acids and lipids. Moreover, cellular structures called cell organelles are suspended in the cytoplasm.

What is a Cell?

A cell is the structural and fundamental unit of life. The study of cells from its basic structure to the functions of every cell organelle is called Cell Biology. Robert Hooke was the first Biologist who discovered cells.

All organisms are made up of cells. They may be made up of a single cell (unicellular), or many cells (multicellular). Mycoplasmas are the smallest known cells. Cells are the building blocks of all living beings. They provide structure to the body and convert the nutrients taken from the food into energy.

Cells are complex and their components perform various functions in an organism. They are of different shapes and sizes, pretty much like bricks of the buildings. Our body is made up of cells of different shapes and sizes.

Cells are the lowest level of organisation in every life form. From organism to organism, the count of cells may vary. Humans have more number of cells compared to that of bacteria.

Cells comprise several cell organelles that perform specialised functions to carry out life processes. Every organelle has a specific structure. The hereditary material of the organisms is also present in the cells.

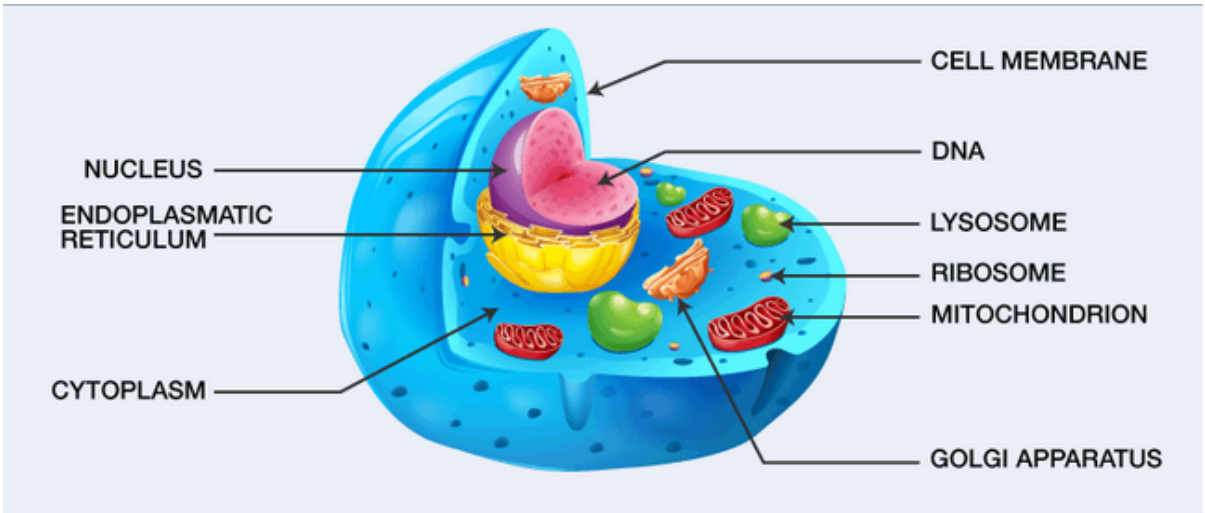


Figure Cell Structure

Cell Structure

The cell structure comprises individual components with specific functions essential to carry out life's processes. These components include- cell wall, cell membrane, cytoplasm, nucleus, and cell organelles. Read on to explore more insights on cell structure and function.

Cell Membrane

- The cell membrane supports and protects the cell. It controls the movement of substances in and out of the cells. It separates the cell from the external environment. The cell membrane is present in all the cells.
- The cell membrane is the outer covering of a cell within which all other organelles, such as the cytoplasm and nucleus, are enclosed. It is also referred to as the plasma membrane.
- By structure, it is a porous membrane (with pores) which permits the movement of selective substances in and out of the cell. Besides this, the cell membrane also protects the cellular component from damage and leakage.
- It forms the wall-like structure between two cells as well as between the cell and its surroundings.
- Plants are immobile, so their cell structures are well-adapted to protect them from external factors. The cell wall helps to reinforce this function.

Cell Wall

- The cell wall is the most prominent part of the plant's cell structure. It is made up of cellulose, hemicellulose and pectin.
- The cell wall is present exclusively in plant cells. It protects the plasma membrane and other cellular components. The cell wall is also the outermost layer of plant cells.
- It is a rigid and stiff structure surrounding the cell membrane.

- It provides shape and support to the cells and protects them from mechanical shocks and injuries.

Cytoplasm

- The cytoplasm is a thick, clear, jelly-like substance present inside the cell membrane.
- Most of the chemical reactions within a cell take place in this cytoplasm.
- The cell organelles such as endoplasmic reticulum, vacuoles, mitochondria, ribosomes, are suspended in this cytoplasm.

Nucleus

- The nucleus contains the hereditary material of the cell, the DNA.
- It sends signals to the cells to grow, mature, divide and die.
- The nucleus is surrounded by the nuclear envelope that separates the DNA from the rest of the cell.
- The nucleus protects the DNA and is an integral component of a plant's cell structure.

Cell Organelles

Cells are composed of various cell organelles that perform certain specific functions to carry out life's processes. The different cell organelles, along with its principal functions, are as follows:

Cell Organelles and their Functions

Nucleolus

The nucleolus is the site of ribosome synthesis. Also, it is involved in controlling cellular activities and cellular reproduction.

Nuclear membrane

The nuclear membrane protects the nucleus by forming a boundary between the nucleus and other cell organelles.

Chromosomes

Chromosomes play a crucial role in determining the sex of an individual. Each human cells contain 23 pairs of chromosomes.

Endoplasmic reticulum

The endoplasmic reticulum is involved in the transportation of substances throughout the cell. It plays a primary role in the metabolism of carbohydrates, synthesis of lipids, steroids and proteins.

Golgi Bodies

Golgi bodies are called the cell's post office as it is involved in the transportation of materials

within the cell.

Ribosome

Ribosomes are the protein synthesisers of the cell.

Mitochondria

The mitochondrion is called “the powerhouse of the cell.” It is called so because it produces ATP – the cell’s energy currency.

Lysosomes

Lysosomes protect the cell by engulfing the foreign bodies entering the cell and help in cell renewal. Therefore, they are known as the cell’s suicide bags.

Chloroplast

Chloroplasts are the primary organelles for photosynthesis. It contains the pigment called chlorophyll.

Vacuoles

Vacuoles store food, water, and other waste materials in the cell.

Functions of Cell

A cell performs major functions essential for the growth and development of an organism. Important functions of cell are as follows:

Provides Support and Structure

All the organisms are made up of cells. They form the structural basis of all the organisms. The cell wall and the cell membrane are the main components that function to provide support and structure to the organism. For eg., the skin is made up of a large number of cells. Xylem present in the vascular plants is made of cells that provide structural support to the plants.

Facilitate Growth Mitosis

In the process of mitosis, the parent cell divides into the daughter cells. Thus, the cells multiply and facilitate the growth in an organism.

Allows Transport of Substances

Various nutrients are imported by the cells to carry out various chemical processes going on inside the cells. The waste produced by the chemical processes is eliminated from the cells by active and passive transport. Small molecules such as oxygen, carbon dioxide, and ethanol diffuse across the cell membrane along the concentration gradient. This is known as passive transport. The larger molecules diffuse across the cell membrane through active transport where the cells require a lot of energy to transport the substances.

Energy Production

Cells require energy to carry out various chemical processes. This energy is produced by the cells through a process called photosynthesis in plants and respiration in animals.

Aids in Reproduction

A cell aids in reproduction through the processes called mitosis and meiosis. Mitosis is termed as the asexual reproduction where the parent cell divides to form daughter cells. Meiosis causes the daughter cells to be genetically different from the parent cells.

Thus, we can understand why cells are known as the structural and functional unit of life. This is because they are responsible for providing structure to the organisms and perform several functions necessary for carrying out life's processes.

UNIT II

13. Elaborate the structural organization and function of protein? (Model Exam 13 Part B QNo. 13)(11 Mark)

What are Proteins?

Proteins are known as the building blocks of life because they are the most abundant molecules present in the body and form about 60% of the dry weight of cells.

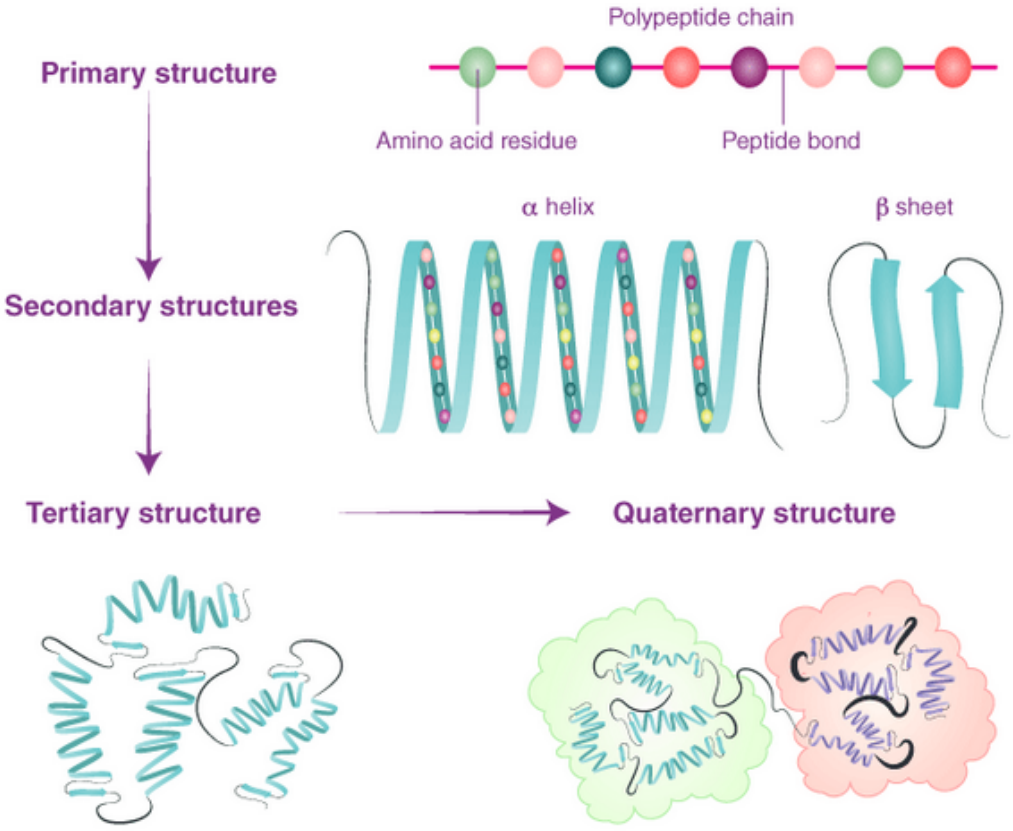


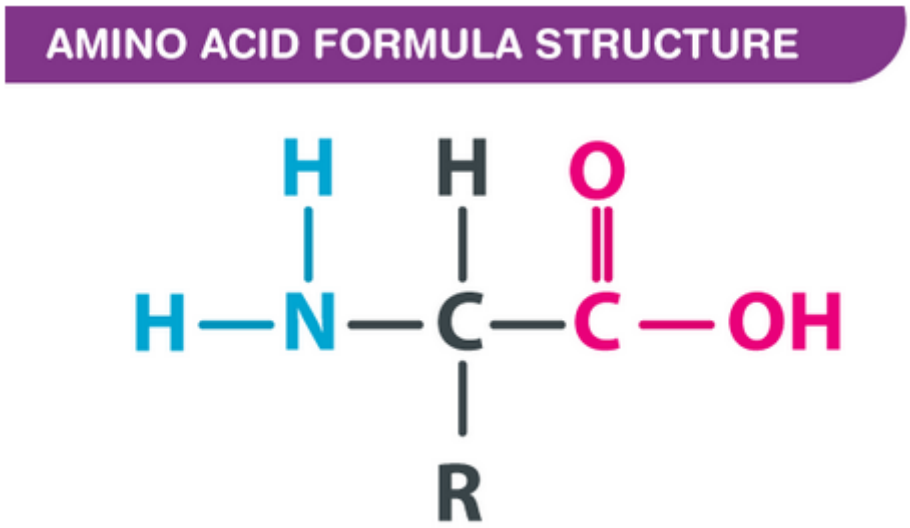
Figure Protein structure

They make up the majority of the cells in all living things. Aside from cells, proteins make up the majority of the body's structural, regulatory, and enzyme components. They are therefore crucial for an individual's growth and development.

Food like eggs, pulses, milk and other milk products form the major high-protein foods for the body.

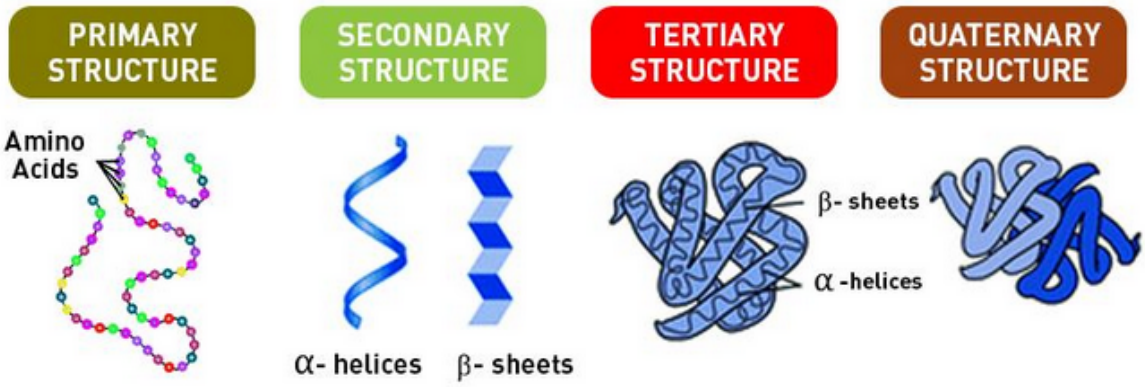
Proteins Structure

A polymeric chain of amino acid residues constitutes proteins. A protein's structure is primarily made up of long chains of amino acids. The arrangement and placement of amino acids give proteins certain characteristics. All amino acid molecules contain an amino (-NH2) and a carboxyl (-COOH) functional group. Hence, the name "Amino-Acid".



Polypeptide chains are synthesized by linking together amino acids. A protein is created when one or more of these chains fold in a specific way. Methane is substituted by amino acids, with hydrogen, amino groups, carboxyl groups, and a variable R- group filling the first three valencies of the – alpha carbon.
There are many sorts of amino acids depending on the R-group, and a polypeptide chain contains 20 of them. The final structure and purpose of proteins are determined by all these characteristics of amino acids.

The structure of the protein is classified at 4 levels:-



Primary – The primary structure of a protein is the linear polypeptide chain formed by the amino acids in a particular sequence. Changing the position of even a single amino acid will result in a different chain and hence a different protein.

- Secondary – The secondary structure of a protein is formed by hydrogen bonding in the polypeptide chain. These bonds cause the chain to fold and coil in two different conformations known as the α-helix or β-pleated sheets. The α-helix is like a single spiral and is formed by hydrogen bonding between every fourth amino acid. The β-pleated sheet is formed by hydrogen bonding between two or more adjacent polypeptide chains.
- Tertiary – The tertiary structure is the final 3-dimensional shape acquired by the polypeptide chains under the attractive and repulsive forces of the different R-groups of each amino acid. This is a coiled structure that is very necessary for protein functions.
- Quaternary – This structure is exhibited only by those proteins which have multiple polypeptide chains combined to form a large complex. The individual chains are then called subunits.

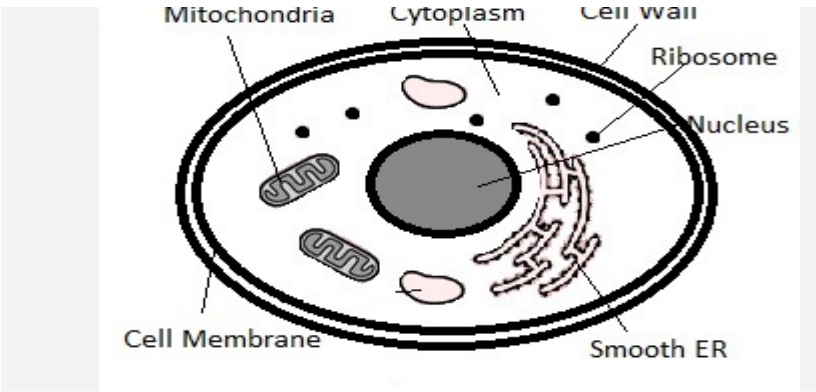
Functions of Proteins

The body uses proteins for a variety of purposes, and their structure determines how they work. Several notable functions include:

1. Digestion – The digestive enzymes, which are primarily proteinaceous in origin, carry out digestion.
2. Movement – Muscles include a protein called myosin, which helps muscles contract, allowing for movement.
3. Structure and Support – The structural protein known as keratin is what gives humans and other animals hair, nails, and horns.
4. Cellular communication – Through receptors on their surface, cells can communicate with other cells and the outside world. These receptors are made of proteins.
5. Act as a messenger – These proteins serve as chemical messengers that facilitate communication among cells, tissues, and organs.

(or)

14. Explain in detail about the process of Protein/Synthesis? (11 mark)



Site of Protein Synthesis in the Cell

Protein synthesis is a group of events resulting in the synthesis of protein molecules. This collection of events include amino acid collection and synthesis, transcription, translation, and post-translational events. In amino acid synthesis, there is a set of biochemical processes that synthesize amino acids from molecules like glucose. All amino acids are not synthesized by the body. Some are obtained from food. Proteins are synthesized in the cells through the transcription and translation processes.

In short, the transcription process involves the creation of mRNA from the DNA template. The translation follows transcription where the amino acids are attached in a specific order with the help of the genetic code. After the translation, the protein that is newly created gets processed further by proteolysis, post-translational modification, further followed by protein folding.

Protein synthesis definition

Protein synthesis is fundamentally the synthesis or creation of proteins that happens inside the cell. This entire process takes place in the cytoplasm in the case of prokaryotes. In eukaryotes, it occurs in the nucleus to generate a transcript, or mRNA, that is synthesized from the coding region of the DNA template strand. The transcript exits the nucleus and approaches the ribosomes to get translated into proteins. This process is aided by tRNA molecules and amino acids.

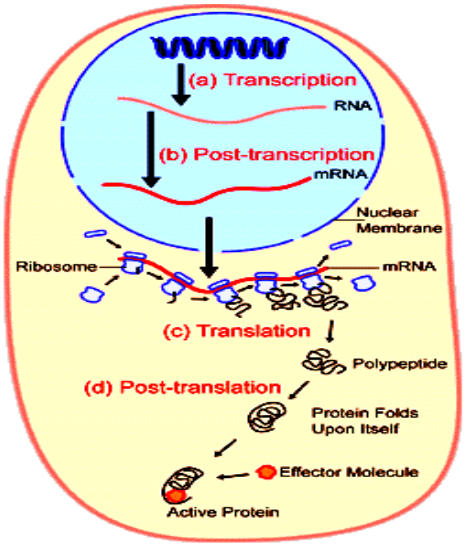


Figure Protein/Synthesis

Site of Protein Synthesis in the Cell

Ribosomes act as the site for protein synthesis. The mRNA molecules travel out of the nucleus into the cytoplasm and are translated into proteins in the cytoplasm. Ribosomes are RNA-protein complexes and are not specific cell organelles. After leaving the nucleus, a few mRNA molecules reach specific sites of the cell before they are translated.

Ribosomes

The structure of ribosomes is similar in both the cells of prokaryotes and eukaryotes. Ribosomes are organized into a large subunit and a small subunit. The ribosomes in prokaryotes consist of nearly 50 different types of protein. Eukaryotes consist of more than 80 different types of proteins. The large subunit consists of two different ribosomal RNAs in prokaryotes and three RNAs in eukaryotes. The small subunit consists of one ribosomal RNA.

The subunit size of ribosomes and the component ribosomal RNAs are measured by the sedimentation coefficient, S which stands for Svedberg units. The Svedberg unit depends on the relative molecular mass and shape. The small and large subunits of prokaryotic ribosomes are the 30S and 50S respectively and of eukaryotic ribosomes are 40S and 60S respectively. The entire ribosome is called the 70S in prokaryotes and the 80S in eukaryotes.

The 30S subunit constitutes 16S rRNA and 21 proteins while the 50S subunit constitutes 23S rRNA, 5S rRNA, and 34 proteins in prokaryotes. In eukaryotes, the 40S subunit constitutes 18S rRNA and 33 proteins while the 50S subunit constitutes 28S rRNA, 5.8S rRNA, 5S rRNA, and 50 proteins.

Genetic Code

A codon represents one group of three nucleotides referring to one amino acid. For example, GCC or guanine-cytosine-cytosine codes for alanine. One of the stop codons out of the three

is UAA (uracil-adenine-adenine). The mRNA codon that is complementary to that of the trinucleotide site of tRNA is called the anticodon.

Roles of mRNA, tRNA, and rRNA

The three types of RNA that are associated with protein synthesis are mRNA, tRNA, and rRNA. The mRNA molecule consists of the code that is relevant to synthesize a protein. The mRNA is generated inside the nucleus and consists of 5' cap, 5' UTR region, coding region, 3' UTR region, and poly(A) tail. The DNA segment copy has a coding region for gene expression. The coding region starts from the start codon at the 5' end and ends with a stop codon at the 3' end.

The tRNA transfers specific amino acids to the ribosome which has to be further added to the growing chain of amino acids. The tRNA consists of two major sites called the anticodon arm and the acceptor stem. The codon of mRNA is complementary to the anticodon site of the tRNA anticodon arm. The acceptor stem is the region where a specific amino acid is attached thereby transforming the tRNA into aminoacyl tRNA. The peptidyl-tRNA will hold the growing polypeptide chain.

rRNA will not have any genetic information and they make up ribosomes. The large subunit acts like a ribozyme that catalyzes the formation of the peptide bond between amino acids. There are three binding sites for rRNA called Aminoacyl, Exit, and Peptidyl sites. The aminoacyl site docks with tRNA, the peptidyl site binds peptide with tRNA, and the

Specialized Cell Structure and Function: Protein Synth

The making of the various types of protein is one of the most important events for a cell because protein not only forms structural components of the cell, it also composes the enzymes that catalyze the production of the remaining organic biomolecules necessary for life. In general, the genotype coded for in the DNA is expressed as a phenotype by the protein and other enzyme-catalyzed products.

The DNA housed in the nucleus is too large to move through the nuclear membrane, so it must be copied by the smaller, single-stranded RNA (transcription), which moves out of the nucleus to ribosomes located in the cytoplasm and rough endoplasmic reticulum to direct the assembly of protein (translation). The genes do not actually make the protein, but they provide the blueprint in the form of RNA, which directs the protein synthesis.

Transcription

Transcription occurs in the cell nucleus and represents the transfer of the genetic code from DNA to a complementary RNA. The enzyme RNA polymerase …

- Attaches to and unzips the DNA molecule to become two separate strands.
- Binds to *promoter* segments of DNA that indicate the beginning of the single strand of DNA to be copied.
- Moves along the DNA and matches the DNA nucleotides with a complementary RNA nucleotide to create a new RNA molecule that is patterned after the DNA.

The copying of the DNA continues until the RNA polymerase reaches a *termination signal*, which is a specific set of nucleotides that mark the end of the gene to be copied and also signals the disconnecting of the DNA with the newly minted RNA.

Translation is

Translation is the conversion of information contained in a sequence of mRNA nucleotides into a sequence of amino acids that bond together to create a protein. The mRNA moves to the *ribosomes* and is "read" by tRNA, which analyzes sections of three adjoining nucleotide sequences, called *codons*, on the mRNA and brings the corresponding amino acid for assembly into the growing polypeptide chain. The three nucleotides in a codon are specific for a particular amino acid. Therefore, each codon signals for the inclusion of a specific amino acid, which combines in the correct sequence to create the specific protein that the DNA coded for.

The assembly of the polypeptide begins when a ribosome attaches to a *start codon* located on the mRNA. Then tRNA carries the amino acid to the ribosomes, which are made of rRNA and protein and have three bonding sites to promote the synthesis. The first site orients the mRNA so the codons are accessible to the tRNA, which occupy the remaining two sites as they deposit their amino acids and then release from the mRNA to search for more amino acids. Translation continues until the ribosome recognizes a codon that signals the end of the amino acid sequence. The polypeptide, when completed, is in its primary structure. It is then released from the ribosome to begin contortions to configure into the final form to begin its function.

UNIT III

15. Elaborate the production of monoclonal antibodies.(Model Part B QNO. 15) (11 Mark)

What is monoclonal antibody production? Monoclonal antibodies are a series of identical antibodies produced by a single clone of B cell. In 1975, monoclonal antibodies were first generated by Milstein and Köhler. This method for production of monoclonal antibodies is called hybridoma technology. Since then, monoclonal antibodies have been widely used as an essential tool of biomedical research and therapeutic applications.

Another method of manufacturing monoclonal antibodies is by using phage display which was discovered by G. Smith in 1985. And it has become one of the most effective techniques for producing large amounts of peptides, proteins and antibodies.

Based on hybridoma and phage display technology, Sino Biological has the capability to offer custom monoclonal antibody services as well as large scale antibody production and purification. Whatever your specifications are, Sino Biological can propose tailored solutions and its expertise to successfully complete each project.

What are the advantages of monoclonal antibodies?

• Same quality of the antibody is maintained amongst the different production batches.
• Highly reproducible and scalable, unlimited production source.
• Speed and sensitivity and specificity of assays.
• Can produce antibodies when needed.
• No need to worry about maintaining the animals.
• Antigen or immunogen need not be pure.
• Selection helps to identify the right clones against the specific antigen.

Steps to produce Monoclonal antibodies.

Some assays require better antibody specificity and affinity than can be obtained using a polyclonal antiserum. To achieve this high specificity, all of the antibodies must bind with high affinity to a single epitope. This high specificity can be provided by monoclonal antibodies (mAbs). Production of monoclonal antibodies involves several critical procedures as follows.

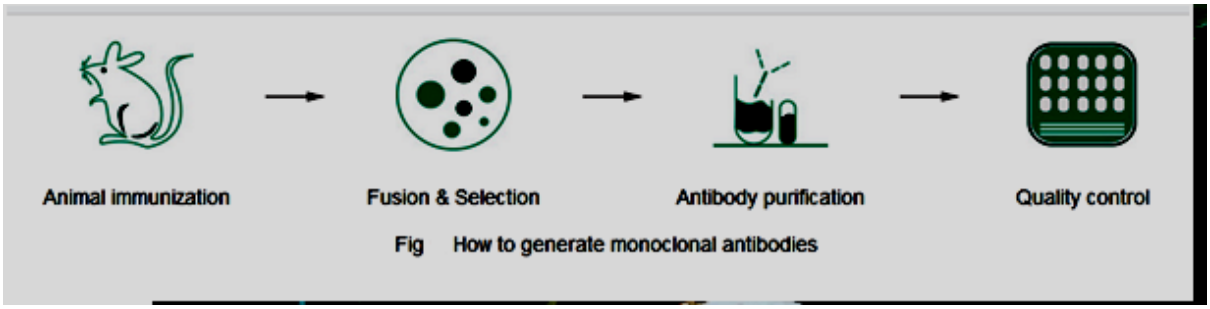


Fig How to generate monoclonal antibodies

1) Animal immunization

The very first step in hybridoma technology is to immunize an animal (usually a mouse), with appropriate antigen. The antigen, along with an adjuvant like Freund's complete or incomplete adjuvant is injected subcutaneously (adjuvants are non-specific potentiators of specific immune responses). The injections at multiple sites are repeated several times.

This enables increased stimulation of B-lymphocytes which are responding to the antigen. Three days prior to killing of the animal, a final dose of antigen is intravenously administered. The immune-stimulated cells for synthesis of antibodies have grown maximally by this approach. The concentration of the desired antibodies is assayed in the serum of the animal at frequent intervals during the course of immunization.

When the serum concentration of the antibodies is optimal, the animal is sacrificed. The spleen is aseptically removed and disrupted by mechanical or enzymatic methods to release the cells. The lymphocytes of the spleen are separated from the rest of the cells by density gradient centrifugation.

2) Fusion & Selection

The thoroughly washed lymphocytes are mixed with HGPRT defective myeloma cells. The mixture of cells is exposed to polyethylene glycol (PEG) for a short period (a few minutes),

since it is toxic. PEG is removed by washing and the cells are kept in a fresh medium. These cells are composed of a mixture of hybridomas (fused cells), free myeloma cells and free lymphocytes.

When the cells are cultured in HAT medium, only the hybridoma cells grow, while the rest will slowly disappear. This happens in 7-10 days of culture. Selection of a single antibody producing hybrid cells is very important. This is possible if the hybridomas are isolated and grown individually. The suspension of hybridoma cells is so diluted that the individual aliquots contain on an average one cell each. These cells, when grown in a regular culture medium, produce the desired antibody.

3) Antibody purification

Monoclonal antibodies may need to be purified before they are used for a variety of purposes. Protein A affinity chromatography is used to purify monoclonal antibodies. It's the golden standard for monoclonal antibody (mAb) purification, and a technology that has gained high interest because of its great performance and capabilities. If customers need higher purity, further purification methods can be performed.

4) Quality control

After purification, a series of quality control tests are performed to ensure the quality of monoclonal antibodies. Antibody concentration is evaluated by absorption at 280 nm (A280). The purity of monoclonal antibody is checked using SDS-PAGE. To estimate the monoclonal antibody titer, an ELISA test was the most suitable method.

(or)

16. Write in detail about the three types of composting.(11 MarK)

Composting Methods

Traditional backyard composting is typically achieved by:

1. Open air composting (hot composting)
2. Direct Composting (in-ground composting)

More Recent methods of composting are:

3. Tumbler Composting (A form of hot composting)
4. Worm Farm Composting (Vermicomposting)
5. EMO Composting (Bacteria composting)
6. Combination Composting (Compot Composting)
7. Commercial Composting
8. Mechanical Composting

Elements generally required in most systems in order to produce compost.

Air	Compost needs to be aerated or it creates an anaerobic environment for bacteria which produces unpleasant odours and attracts vermin
Water	Essential to keep the compost moist
Vegetable Matter	Essential to obtain organically rich compost
Worms	Digest decomposed matter and release worm castings that provide plants with the nutrients they need for growth
Carbon-nitrogen mix (brown and green waste)	Essential to create the right temperature for creating compost from green waste and to kill seeds and disease
Bacteria (EMO's)	Will decompose the food before the worms eat it
Soldier Flies	Not essential but devours waste food quicker than worms or bacteria
Other Beneficial Bugs	Cockroaches and other insects that help in the decomposition process (including maggots if putting meat in a compost pile – not recommended for most composters except the Compot.

1. Open Air Composting

Open Air Composting is traditionally a pile of green and brown matter in your backyard.

Open Air Composting is generally considered to be a Hot Composting method. Some people often call it a Cold Composting when smaller quantities of waste are used because it does not build up the same amount of heat.

To me, Cold Composting still produces heat and therefore is not technically cold composting.

Perhaps one could call it Warm Composting as the only way you could completely cold compost something is to let it rot in the fridge. And we all know that smell in the fridge.

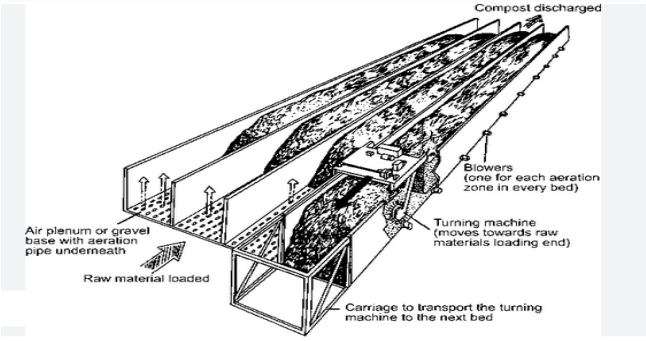


Figure Open Air Composting

2. Direct Composting

Direct Compost is simply digging a hole or trench in the ground and burying your scraps.

It is also probably the oldest and most effective method of composting, but like all other methods of composting it too has its limitations. The main one being that it takes a long time to decompose unless you chop everything up.

You can only bury fruit and veg or you run the risk of it being dug up by all sorts of garden critters from birds to vermin. And you have to keep digging holes.

It does, however, produce an abundance of worms that then help to nourish your garden and improve your soil.

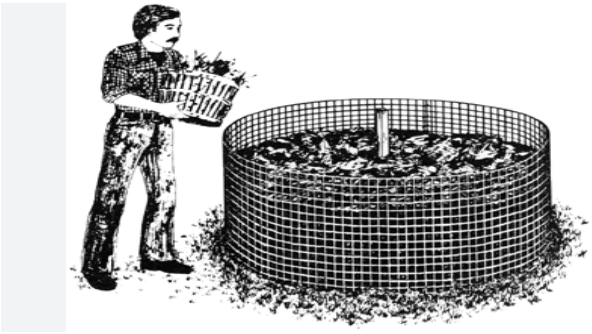


Figure Direct Composting by digging a hole or trench

3. Tumbler Composting

Tumbler Composting comes in many shapes and sizes of single to double units that you may purchase commercially from your local hardware store or home made.

For many people, this is a great system if you are relatively strong and keen to turn it every day or every few days.

Figure Tumbler Composting

4. Worm Farm Composting

Worm Farm Composting for many, is the most common and preferred choice of composting because of their capabilities to grow worms, produce compost and compost tea and keep rats out of your compost.

The worms produce castings concentrated with nutrients lower in nitrogen compared to other composting methods.

Figure Worm Farm Composting

5. EMO Composting

EMO Composting or Effective Microorganisms is a system generally used for indoor composting but can be used by anyone who likes this method of composting or perhaps lives in a unit.

The most common product using EMO's is the Bokashi but other indoor systems can use it plus there are some systems that use a carbon filter in the lid as well to filter odors.

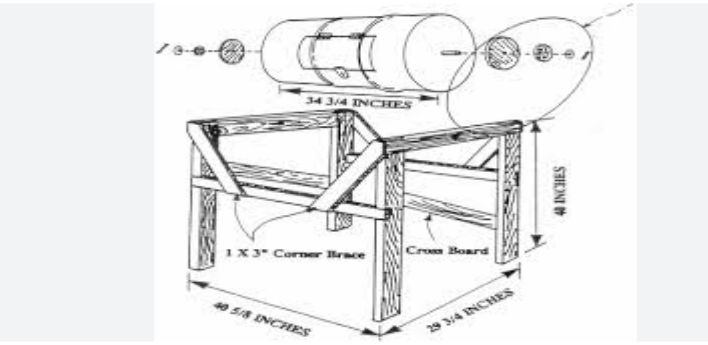


Figure EMO Composting

6. Combination Composting

Combination Composting or Compot Composting is a combination method of open-air composting, direct composting, vermicomposting, and EMO composting.

Figure Combination Composting

7. Commercial Composting

Commercial Composting is different to backyard composting and uses different materials. The Compost is made in long rows using such materials as, sawdust, pine bark, sand plus ferrous sulphate and maybe some sulphate of ammonia all mixed together.

Figure Commercial Composting

8. Mechanical Composting

Mechanical Composting is an efficient method of composting that uses electricity to create the heat required and rotation of the contents required to produce semi-composted waste literally within a 24 hour period.

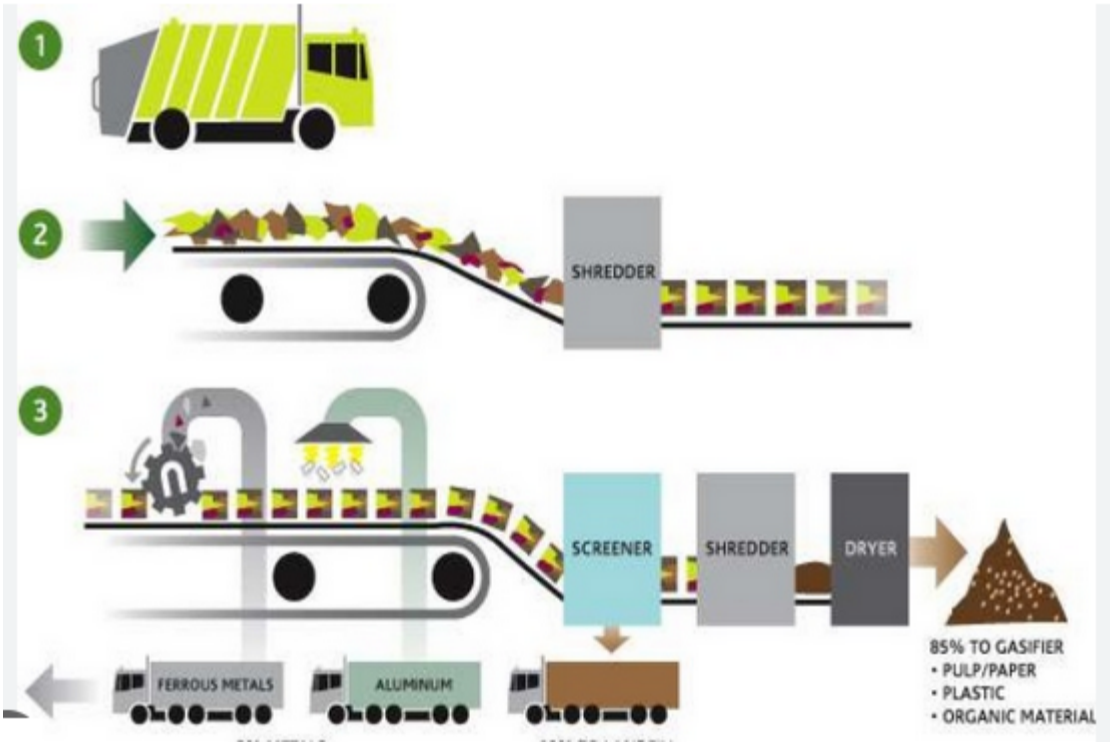


Figure Mechanical Composting

UNIT IV

17. Describe the steps involved in recombinant insulin production.(11 Mark)

Recombinant DNA is a technology scientists developed that made it possible to insert a human gene into the genetic material of a common bacterium. This “recombinant” micro-organism could now produce the protein encoded by the human gene.

The steps in the production of human insulin by genetic engineering method includes:

1. Human insulin is extracted from pancreas cells and an insulin-producing gene is isolated.

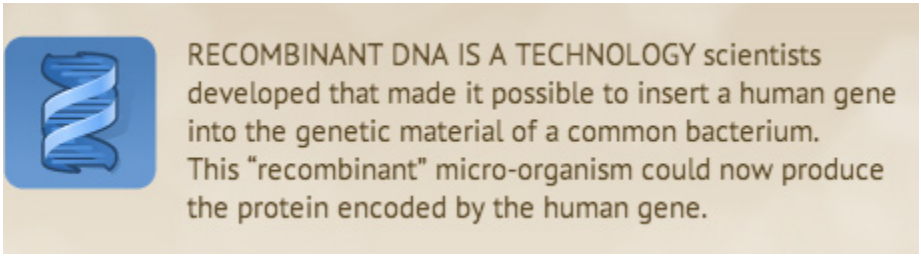
2. A plasmid DNA is extracted from a bacterium and cut with restriction enzyme, forming plasmid vector.

3. Insert human insulin-producing gene into the bacterial plasmid vector to form the recombinant DNA of human insulin-producing gene.

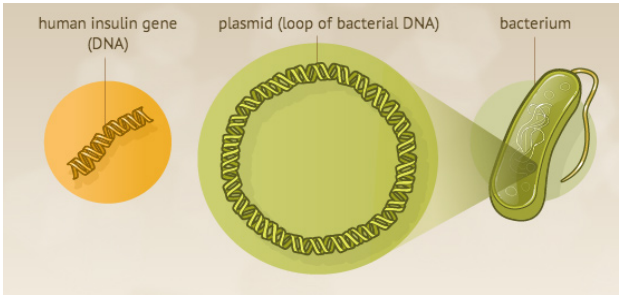
4. Introduce this recombinant DNA into a bacterial cell to form the recombinant bacterium.

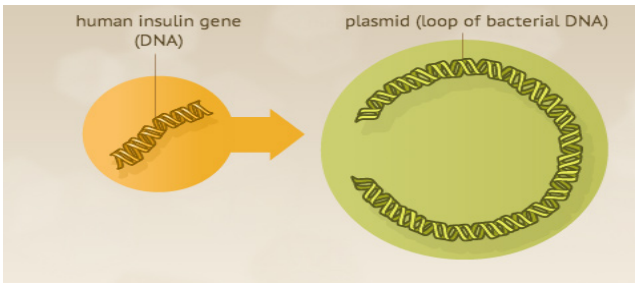
5. The recombinant bacteria multiply in a fermentation tank and produce human insulin.

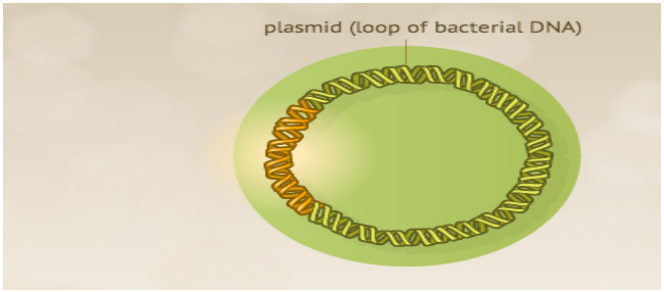
6. Insulin is extracted, purified and bottled. It is then ready to be injected into diabetic patients.

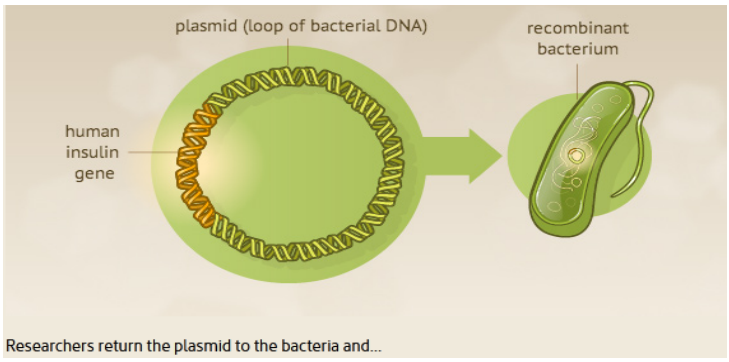


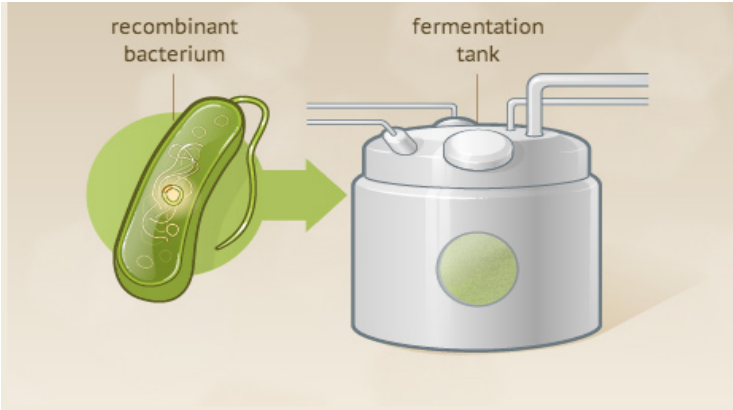
Scientists build the human insulin gene in the laboratory. Then they remove a loop of bacterial DNA known as a plasmid and nsert the human insulin gene into the plasmid.



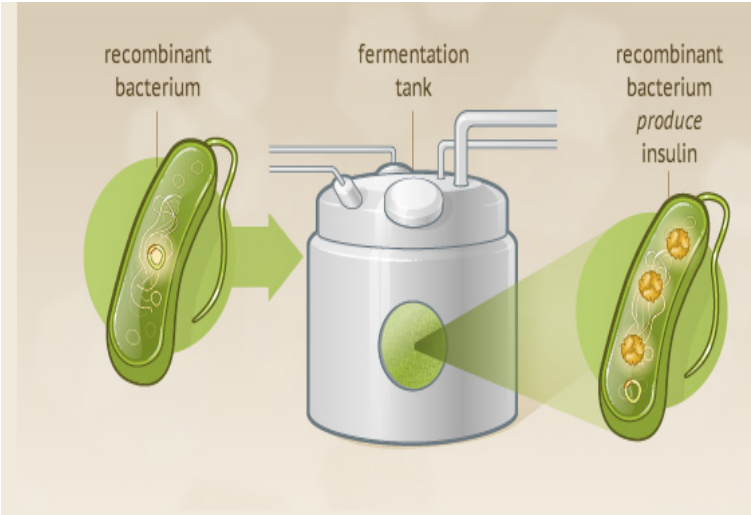




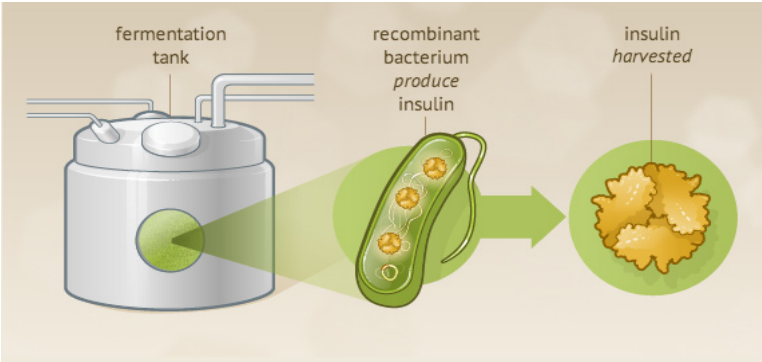




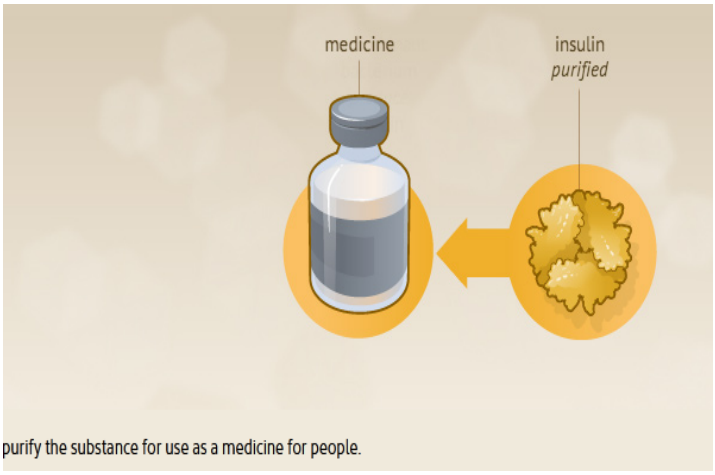
put the "recombinant" bacteria in large fermentation tanks.



There, the recombinant bacteria use the gene to begin producing human insulin.



Scientists harvest the insulin from the bacteria and...



The steps involved in production of insulin are:

1. Isolation of desired gene: Human insulin gene is isolated from human tissue. The cells are removed and cultured. The cell is broken and DNA is isolated by breaking the nucleus. The desired gene is obtained by cutting DNA with the help of restriction endonuclease. DNA containing the desired gene is called passenger DNA.
2. Formation of recombinant DNA: Plasmids are usually used as vectors. The plasmid is cleaved open at a site with the help of restriction endonuclease. Now, the desired gene of interest is mixed with the plasmid DNA and joined by means of DNA ligase. The plasmid containing the desired gene is called recombinant DNA.
3. Gene Transfer to host: The recombinant DNA is introduced into a host cell (eg. *E.coli*). When the bacterial cell divided rapidly, the plasmid along with the desired gene is also amplified. Thus, several copies of the desired gene are produced. The desired gene is allowed to produce the desired product i.e. Insulin.

(or)

18. Explain the guidelines in implementing bioethics in lab research. (Internal1 Part B QNo 15)

Guiding Principles for Ethical Research

- Social and clinical value.
- Scientific validity.
- Fair subject selection.
- Favorable risk-benefit ratio.
- Independent review.
- Informed consent.
- Respect for potential and enrolled subjects.

Social and clinical value

Every research study is designed to answer a specific question. The answer should be important enough to justify asking people to accept some risk or inconvenience for others. In other words, answers to the research question should contribute to scientific understanding of

health or improve our ways of preventing, treating, or caring for people with a given disease to justify exposing participants to the risk and burden of research.

Scientific validity

A study should be designed in a way that will get an understandable answer to the important research question. This includes considering whether the question asked is answerable, whether the research methods are valid and feasible, and whether the study is designed with accepted principles, clear methods, and reliable practices. Invalid research is unethical because it is a waste of resources and exposes people to risk for no purpose

Fair subject selection

The primary basis for recruiting participants should be the scientific goals of the study — not vulnerability, privilege, or other unrelated factors. Participants who accept the risks of research should be in a position to enjoy its benefits. Specific groups of participants (for example, women or children) should not be excluded from the research opportunities without a good scientific reason or a particular susceptibility to risk.

Favorable risk-benefit ratio

Uncertainty about the degree of risks and benefits associated with a clinical research study is inherent. Research risks may be trivial or serious, transient or long-term. Risks can be physical, psychological, economic, or social. Everything should be done to minimize the risks and inconvenience to research participants to maximize the potential benefits, and to determine that the potential benefits are proportionate to, or outweigh, the risks.

Independent review

To minimize potential conflicts of interest and make sure a study is ethically acceptable before it starts, an independent review panel should review the proposal and ask important questions, including: Are those conducting the trial sufficiently free of bias? Is the study doing all it can to protect research participants? Has the trial been ethically designed and is the risk–benefit ratio favorable? The panel also monitors a study while it is ongoing.

Informed consent

Potential participants should make their own decision about whether they want to participate or continue participating in research. This is done through a process of informed consent in which individuals (1) are accurately informed of the purpose, methods, risks, benefits, and alternatives to the research, (2) understand this information and how it relates to their own clinical situation or interests, and (3) make a voluntary decision about whether to participate.

Respect for potential and enrolled participants

Individuals should be treated with respect from the time they are approached for possible participation — even if they refuse enrollment in a study — throughout their participation and after their participation ends. This includes:

- respecting their privacy and keeping their private information confidential
- respecting their right to change their mind, to decide that the research does not match their interests, and to withdraw without a penalty
- informing them of new information that might emerge in the course of research, which might change their assessment of the risks and benefits of participating
- monitoring their welfare and, if they experience adverse reactions, unexpected effects, or changes in clinical status, ensuring appropriate treatment and, when necessary, removal from the study
- informing them about what was learned from the research

UNIT V

19. Write in detail about the method of cheese production using microbes.(11 MarK)

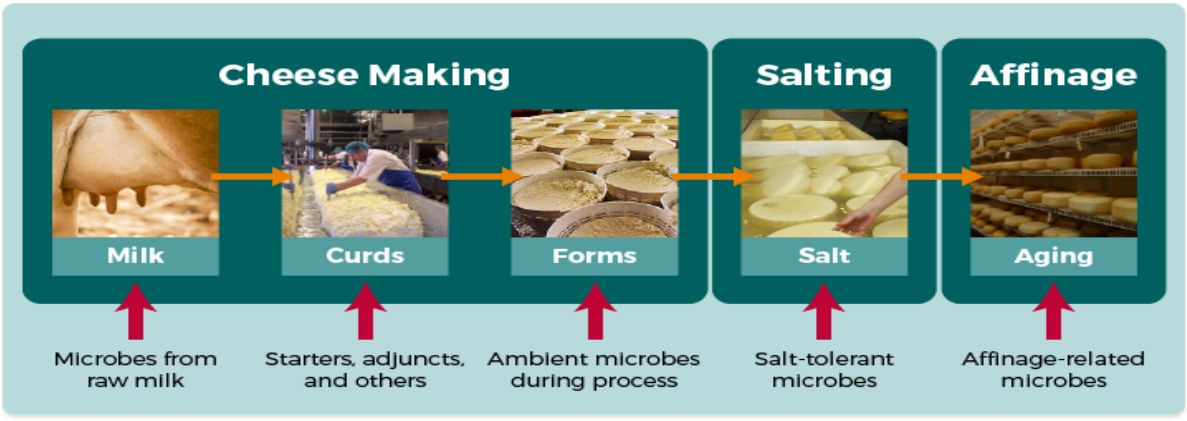
What are Microbes?

"Microbe" is short for microorganism -- any microscopic living organism*. There are many things found in this branch of the evolutionary tree. In cheese we usually find bacteria, yeasts, and molds. Even looking at just these categories leaves thousands upon thousands of microbes that could potentially be found cheese. For brevity's sake, we're going to cover just the basics (thus the "101"). There is a lot we won't cover here. If you're looking for a more in-depth review of microbes and cheese, check our MicrobialFoods.org.

*To avoid excessive repetition we'll be using "microbe", "organism", and "bug" interchangeably. I'll try my best to get the spelling right and not say how important orgasms are to cheese making.

Microbes in Cheese

The bacteria, molds, yeasts, etc. that find their way into cheese can be added intentionally by the cheesemaker or affineur. And by intentionally, I mean a person made a judgement call and chose which organism to add to the cheese. Of more interest and import are the multitude of microbes that are introduced into the cheese without any direct decision making from the cheesemaker/affineur. This is where a cheese takes on its so-called "terroir". Microbes native to the milk will be carried over to the cheese and as cheese is being made and as it is being aged there are many ambient organisms that weasel themselves i



Microbes are introduced into cheese at every step in the cheese making process

Lactic Acid Bacteria

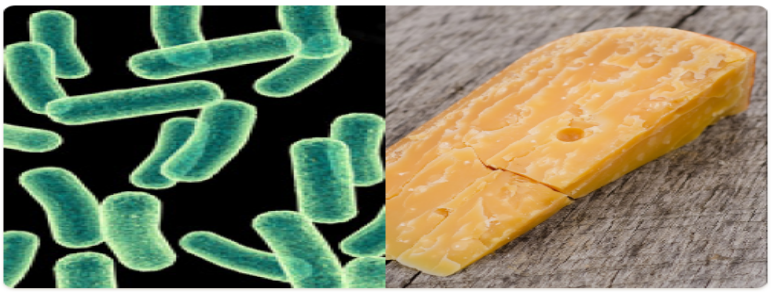
These are the microbes (bacteria) that are added to the milk very early in the cheese making process that induce the fermentation process. The main reaction taking place here is the conversion of lactose to lactic acid, acidifying the milk, which explains how they get their name. You may have also heard of these guys referred to as "starter cultures". Examples from this category include:

- Lactococci - *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* are common lactic acid bacteria that are used to make cheeses like cheddar
- Streptococci - *Streptococcus salivarius* ssp. *thermophilus* is an example of a culture used in cheese like mozzarella
- Lactobacilli - *Lactobacillus helveticus* is an example of a culture commonly used in Swiss and alpine cheeses. *L. helveticus* is also commonly used as an adjunct. (below)

Lactic acid bacteria are a ubiquitous starter culture

Adjunct Cultures

Adjuncts are microbes that are added for reasons other than just producing lactic acid. In many cases, adjuncts are added to encourage flavor development in the cheese. *Lactobacillus helveticus* (see above) is a common example, often giving cheeses a pleasant sweet flavor and promoting the growth of tyrosine crystals.



Lactobacillus helveticus often gives a sweet flavor to cheeses like aged Gou

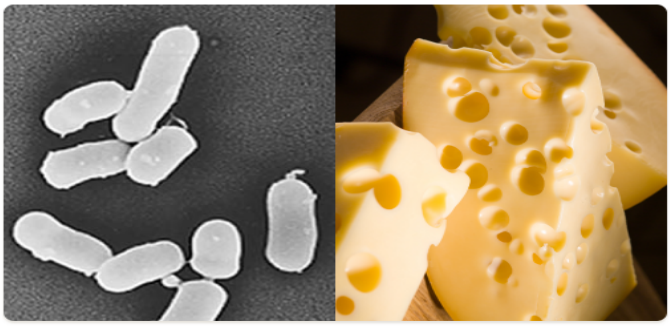
NSLAB

Related to adjuncts, Non-Starter Lactic Acid Bacteria are lactic acid bacteria that grow as cheese is ripened that weren't added for the express purpose of acidifying the milk. Usually these microbes are present naturally in the milk or get picked up along the way during cheesemaking. As cheese ages, the numbers of NSLAB increase while starter cultures die off. Each of their exact roles in cheese flavor development is still trying to be understood completely. Examples include:

- *Lactobacillus casei* ssp. *casei*
- *Lactobacillus plantarum*

Eye Formers

Swiss cheese (and Gouda to a lesser degree) have pronounced eye (hole) formation due to the action of certain bacteria. *Propionibacterium freudenreichii* ssp. *shermanii* is a specific bacterium that converts lactic acid into carbon dioxide, propionic acid, and acetic acid. The carbon dioxide seeps into the cheese body and produces the eyes we all know and love. The other products of Propionibacterium metabolism also give the characteristic flavors commonly associated with Swiss cheeses.



Propionibacterium produce CO_2 and form the eyes in Swiss

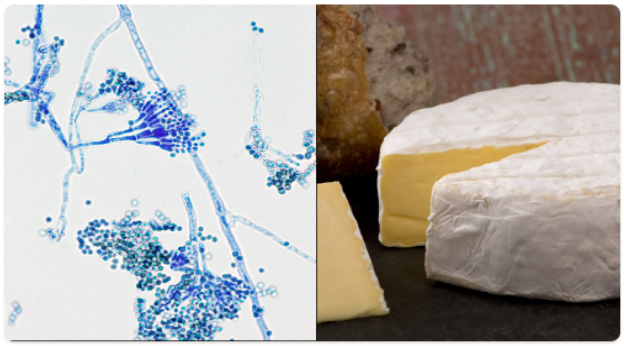
Gouda cheese often have eyes as well, although usually to a smaller degree than Swiss. In this case, it's not propionibacteria that is responsible, but usually bacteria such as *Leuconostoc mesenteroides* and *Lactococcus lactis* ssp *lactis* biovar. *diacetylactis*. In this case it is citric acid that is converted to carbon dioxide and diacetyl (buttery flavor).

Molds

The two main molds that are found in/on cheese are blue and white, respectively. While there are specific molds cheesemakers add to get the cheese they want, there are many molds that grow naturally on the surfaces of cheese during affinage. We'll be covering the former.

White Mold

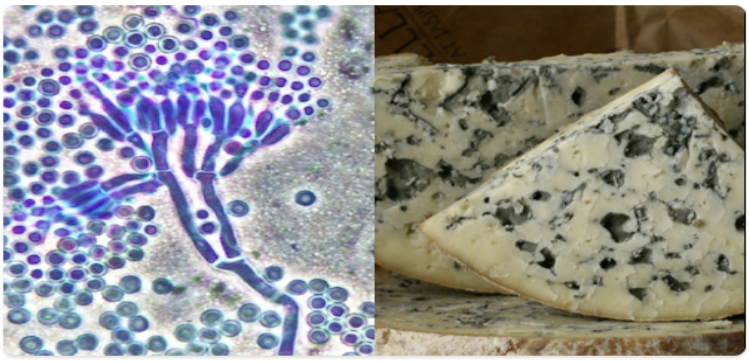
Penicillium camemberti (fka *Penicillium candidum*) is the most popular mold species that is responsible for the nice white lawn on the surfaces of cheeses like camembert and brie. The metabolism of this mold is responsible for some of the characteristic aromas associated with white mold cheeses (mushroom, ammonia, etc.) as well as the texture (soupy goodness). You can often see this by looking at a cross section of a piece of young brie. You'll observe a translucent soupy boarder surrounding a chalky center.



Penicillium camemberti covers Brie and Camembert

Blue Mold

Penicillium roqueforti and *Penicillium glaucum* are the big players in the blue mold worlds. These are what give bleus the blues. The pigments are created by the molds as well as the unique flavor and distinctive texture. As mentioned in the opening paragraph of the post, these molds are living *breathing* organisms. Starving them of oxygen will change their metabolism and they will change color as well as produce off-flavors. For this reason it's best to never vacuum package blue cheeses (or any mold-contain cheese for that matter). A common misconception is that when blue cheeses are pierced during the aging process that mold is being injected. Actually, those needles are there to create air channels. The mold is usually added to the milk during the preliminary cheese making steps or to the curds before they're hooped. The piercing only serves to encourage mold growth by introducing oxygen



Penicillium roqueforti is a common blue mold added to cheese

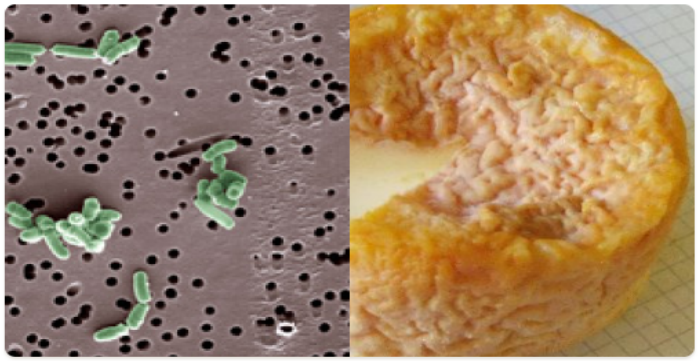
Mold-like Yeast

I'd also like to mention *Geotrichum candidum* which is a yeast that exhibits mold-like tendencies. This microbes is responsible for the "brainy" appearance some cheeses have.

Geotrichum candidum is responsible for the brainy appearance of some cheeses

Surface Ripened Bacteria

Brevibacterium linens is one of the most common bacteria that make up "smear" bacteria. It is also responsible for foot odor, which explains the smell of many surface ripened cheeses. This bacterium produces a multitude of compounds including ones that give rise to the distinctive aroma. *Corynebacteria* are another class of bacteria commonly found on these cheeses. It's important to remember that the combination of microorganisms is what makes the magic happen for many cheeses.



Brevibacterium linens and yeast often produces orange colored pigments

Yeasts

Often forgotten, but yeast are commonly used in the molded and surface ripened cheeses. They are also naturally present in many natural rind cheeses. These are important parts of the aging process of many cheeses. In many cases, there is a careful balance of yeast, mold, and bacteria that give rise to natural rinds. This will have its own post one day.

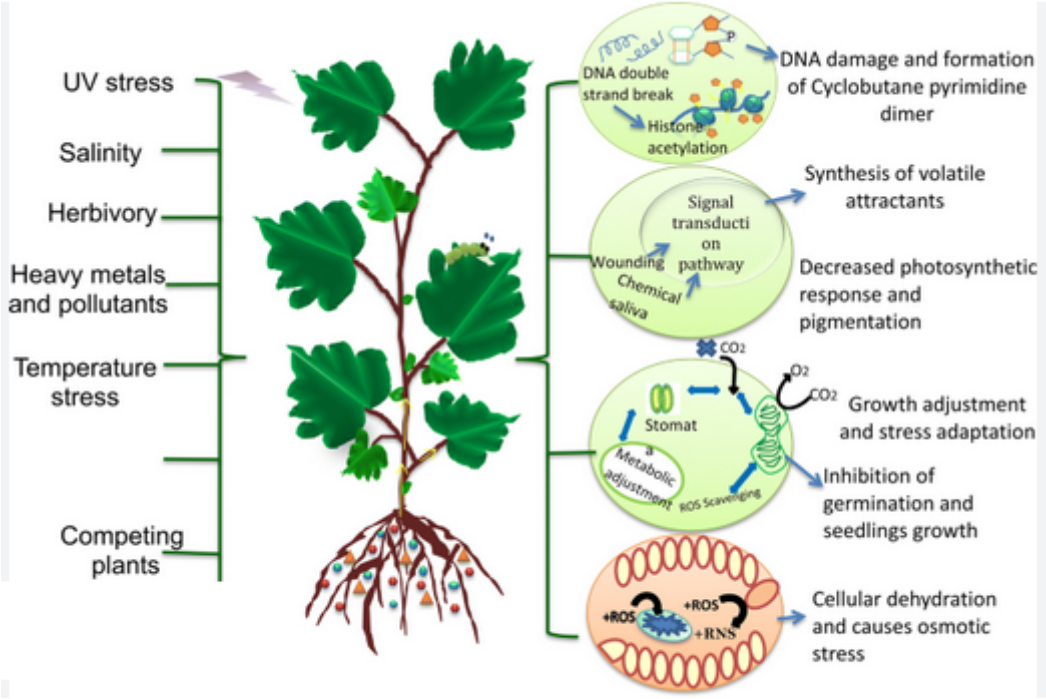
(or)

20. Explain the detail about the secondary metabolites of plants and mention its application.

Secondary metabolism produces a large number of specialized compounds (estimated 200,000) that do not aid in the growth and development of plants but are required for the plant to survive in its environment. Secondary metabolism is connected to primary metabolism by using building blocks and biosynthetic enzymes derived from primary metabolism. Primary metabolism governs all basic physiological processes that allow a plant to grow and set seeds, by translating the genetic code into proteins, carbohydrates, and amino acids. Specialized compounds from secondary metabolism are essential for communicating with other organisms in mutualistic (e.g. attraction of beneficial organisms such as pollinators) or antagonistic interactions (e.g. deterrent against herbivores and pathogens). They further assist in coping with abiotic stress such as increased UV-radiation. The broad functional spectrum of specialized metabolism is still not fully understood. In any case, a good balance between products of primary and secondary metabolism is best for a plant's optimal growth and development as well as for its effective coping with often changing environmental conditions. Well known specialized compounds include alkaloids, polyphenols including flavonoids, and terpenoids. Humans use many of these compounds for culinary, medicinal and nutraceutical purposes.

secondary plant metabolism

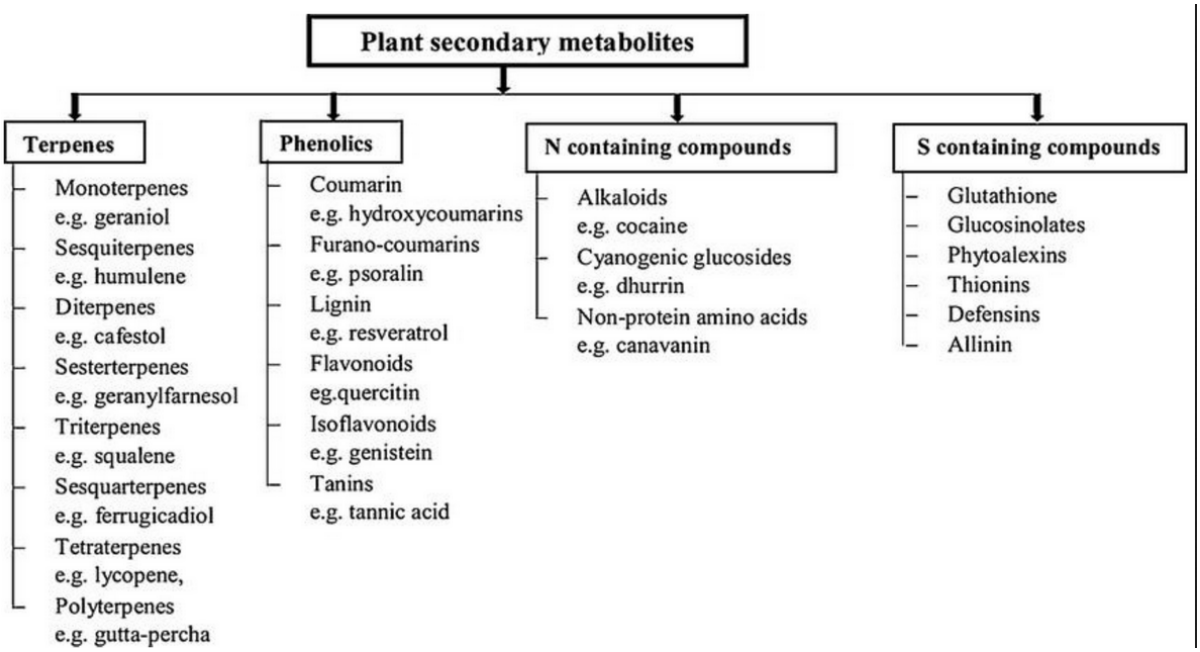
Secondary plant metabolites are also used in signalling and regulation of primary metabolic pathways. Plant hormones, which are secondary metabolites, are often used to regulate the metabolic activity within cells and oversee the overall development of the plant. As mentioned above in the History tab, secondary plant metabolites help the plant maintain an intricate balance with the environment, often adapting to match the environmental needs. Plant metabolites that color the plant are a good example of this, as the coloring of a plant can attract pollinators and also defend against attack by animals.



Plant secondary metabolites synthesis

Plant cell cultures represent a potential source of valuable secondary metabolites which can be used as food additives, nutraceuticals, and pharmaceuticals. The synthesis of phytochemicals by the cell cultures in contrast to these in plants is independent of environmental conditions and quality fluctuations.

Secondary plant metabolites are extensively used in today's food industries, for example, as coloring-agents, flavouring-agents or texturizing agents. In particular, metabolites with antioxidative properties find applications as preservatives or anti-browning agents.13-Aug-2018



Many secondary compounds have important adaptive significance in protection against herbivory and microbial infection, as attractants (pigments or scents) for pollinators and seed-dispersing animals, and as allelopathic agents

Secondary metabolites have complex chemical composition and are produced in response to various forms of stress to perform different physiological tasks in plants. They are used in pharmaceutical industries, cosmetics, dietary supplements, fragrances, flavors, dyes

Secondary metabolites have a defense function in plants [1, 2]. The simplest way to utilize secondary metabolites for crop protection is to extract the secondary metabolites and apply them to crops for protection against pathogens, insects, and mammalian herbivores.

APPLICATIONS

- Functional analysis of genes.
- Expressing foreign proteins.
- Production of secondary metabolites.
- May change the composition of metabolites.
- Can be used to regenerate a whole plant.
- May produce compounds which is not found in untransformed roots.
- Quinine for malaria.
  (ii)Shikonin used for anti bacterial and anti ulcer agent.
  (iii)Berberine etc.