Chapter 12

Biotechnology and its Applications

Biotechnological Applications in Agriculture

Biotechnological Applications in Agriculture

Let us take a look at the three options that can be thought for increasing food production
(i) agro-chemical based agriculture;
(ii) organic agriculture; and
(iii) genetically engineered crop-based agriculture.

Fig: Chemicals in Agriculture

The **Green Revolution** succeeded in tripling the food supply but yet it was not enough to feed the growing human population. Increased yields have partly been due to the use of improved crop varieties, but mainly due to the use of better management practices and use of agrochemicals (fertilisers and pesticides). However, for farmers in the developing world, agrochemicals are often too expensive, and further increases in yield with existing varieties are not possible using conventional breeding.
Is there any alternative path that our understanding of genetics can show so that farmers may obtain maximum yield from their fields? Is there a way to minimise the use of fertilisers and chemicals so that their harmful effects on the environment are reduced? Use of genetically modified crops is a possible solution.
Plants, bacteria, fungi and animals whose genes have been altered by manipulation are called Genetically Modified Organisms (GMO). GM plants have been useful in many ways.

Genetic modification

(i) made crops more tolerant to abiotic stresses (cold, drought, salt, heat).
(ii) reduced reliance on chemical pesticides (pest-resistant crops).
(iii) helped to reduce post harvest losses.
(iv) increased efficiency of mineral usage by plants (this prevents early exhaustion of fertility of soil).
(v) enhanced nutritional value of food, e.g., Vitamin 'A' enriched rice.

Fig: Genitic Modification

In addition to these uses, GM has been used to create tailor-made plants to supply alternative resources to industries, in the form of starches, fuels and pharmaceuticals. Some of the applications of biotechnology in agriculture that you will study in detail are the production of pest resistant plants, which could decrease the amount of pesticide used.

Bacillus thuringiensis

Bt toxin is produced by a bacterium called Bacillus thuringiensis (Bt for short). Bt toxin gene has been cloned from the bacteria and been expressed in plants to provide resistance to insects without the need for insecticides; in effect created a bio-pesticide. Examples are Bt cotton, Bt corn, rice, tomato, potato and soyabean etc.

Bt Cotton: Some strains of Bacillus thuringiensis produce proteins that kill certain insects such as lepidopterans (tobacco budworm, armyworm), coleopterans (beetles) and dipterans (flies, mosquitoes).

Bt 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 exists as inactive protoxins but once an insect ingest the inactive toxin, it is converted into an active form of toxin due to the alkaline pH of the gut which solubilises the crystals.

The activated toxin binds to the surface of midgut epithelial cells and creates 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 several crop plants such as cotton. The choice of genes depends upon the crop and the targeted pest, as most Bt toxins are insect-group specific.

The toxin is coded by a gene named cry. There are a number of them, for example, the proteins encoded by the genes cryIAc and cryIIAb control the cotton bollworms, that of cryIAb controls corn borer.

Fig: Cotton ball

Pest Resistant Plants

Several nematodes parasitised a wide variety of plants and animals including human beings. A nematode Meloidegyne incognitia infects the roots of tobacco plants and causes a great reduction in yield.

Fig: Pest Resistant Plants

A novel strategy was adopted to prevent this infestation which was based on the process of RNA interference (RNAi). RNAi takes place in all eukaryotic organisms as a method of cellular defense.

This method involves silencing of a specific mRNA due to a complementary dsRNA molecule that binds to and prevents translation of the mRNA (silencing). The source of this complementary RNA could be from an infection by viruses having RNA genomes or mobile genetic elements (transposons) that replicate via an RNA intermediate.

Using **Agrobacterium** vectors, nematode-specific genes were introduced into the host plant. The introduction of DNA was such that it produced both sense and anti-sense RNA in the host cells.

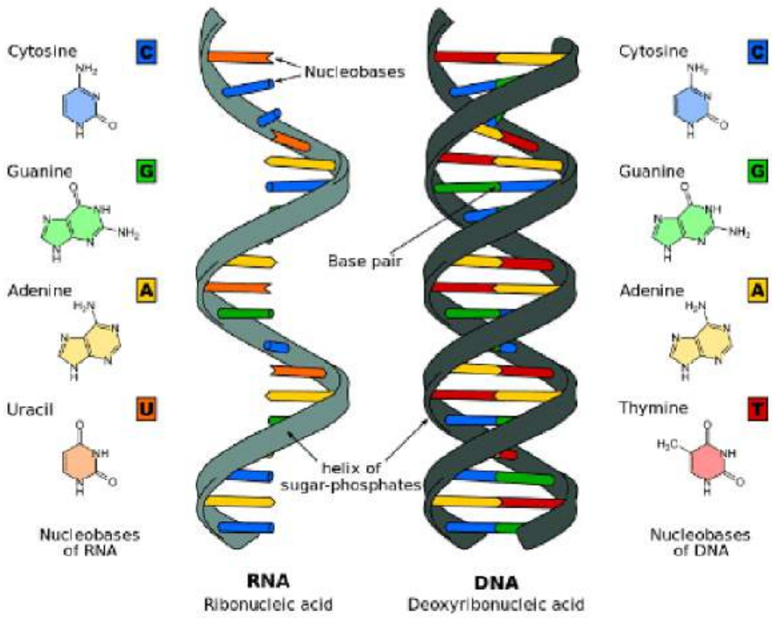


Fig: RNA and DNA

These two RNA's being complementary to each other formed a double stranded (dsRNA) that initiated RNAi and thus, silenced the specific mRNA of the nematode. The consequence was that the parasite could not survive in a transgenic host expressing specific interfering RNA. The transgenic plant therefore got itself protected from the parasite.

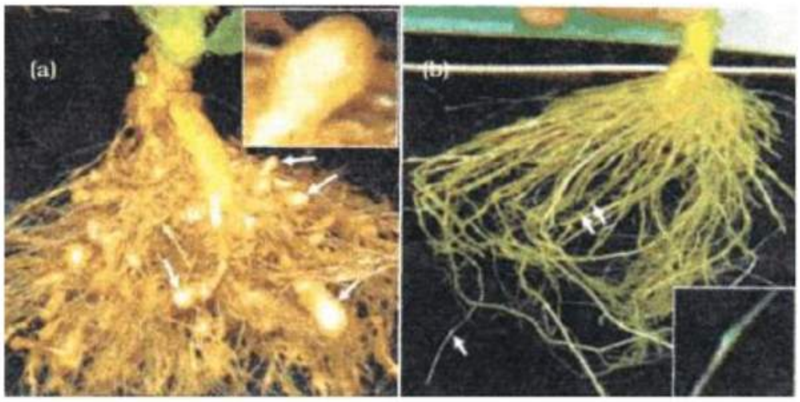


Fig: Host plant-generated dsRNA triggers protection against nematode infestation:
(a) Roots of a typical control plants;
(b) transgenic plant roots 5 days after deliberate infection of nematode but protected through novel mechanism

Biotechnology in Medicine

VITAMINS:

Definition:-"Vitamins are complex organic substances found in various food and required for specific metabolic reaction in cells. "The term vitamin first of all used by **Funk** and first of all **Vit-B1** also isolated by him, **Vit-C** isolated by **A.S.Gyorgy.** The **first vitamin** to be produced by fermentation was V**it-C** by A.S. Gyorgy also **Vit-A** was isolated by **Mc Collum** and **Vit-D** by **Millanby**.
Microbes are important commercial sources of several vitamins. Some examples are given here :-

1. Riboflavin (vitamin B_2):- This found in cereals, vegetables and Brewer's yeast was first produced in 1938. It is a crystalline, bitter, odourless, yellowish brown chemical and is essential for growth and reproduction in animals. The main sources of Riboflavin are **fungi– Ashbya gossypii** and the yeast like **Eremothecium ashybyii**. By the use of original wild strain of mould Ashbya gossypi, production of Vit-B2 increase 100-300 times more.

2. Cobalamin or Vitamin B_{12} :- First isolated from **liver extract** in 1948. It is a compound which contains cobalt, and is now obtained in a cobalt rich substrate (eg. corn sugarcane molasses or starch) by microbes such as **Propionibacterium frendenreichii, Bacillus megatherium**.
Vitamin B_{12} is used to supplement animal feed, and in man for increasing appetite and for treating Anaemia. By the use of Mutant strains of **Pseudomonas denitrificans,** production of Vit-B12 increased 50,000 times more.

3. Ascorbic Acid (Vitamin C) :- It is manufactured from L.Sorbose which is commercially produced from Sorbitol by Biological dehydrogenation brought about by different species of **Acetobacter**.

ANTIBIOTICS

The term was coined by **Selman Waksman** (1942).
Definition:-"An antibiotic is a substance produced by a microorganism which in low concentration inhibits the growth and metabolic activity of pathogenic organism without harming the host."
Alexander Flemming was first to produce and antibiotic named **penicillin** from **Penicillium notatum**.
Waksman and **Albert** discovered **Streptomycin** and **Actinomycin**.
Burkholder isolated **Chloromycetin**.
Antibiotics are of two types :-

1. Broad spectrum Antibiotic :- It is an antibiotic which can kill or destroy a number of pathogens that belong to different groups with different structure and wall composition.

2. Limited spectrum (Specific) antibiotic :- It is an antibiotic which is effective only against one type of pathogens.
Action of Antibiotics :- An antibiotic acts on pathogen by:
(1) disruption of wall synthesis.
(2) Disruption of plasmalemma repair and synthesis.
(3) inhibition of DNA/RNA/Protein synthesis.
Good Antibiotic :-
A. Harmless to host with no side effect.
B. Harmless to normal micro-flora of Alimentary canal.
C. Ability to destroy pathogen as well as broad spectrum.
D. Effective against all strains of pathogen E. Quick Action **Resistance to Antibiotics** :-Pathogens often develop resistance to existing antibiotics so that newer antibiotics are required to be produced.
The resistance is produced due to :-.
i Development of copious mucilage.
ii. Alteration of cell membrane so that antibiotic cannot recognise the pathogen.
iii. Change to L-form by pathogen.
iv. Mutation in pathogen.
Main sources of Antibiotic :-**The main sources of Antibiotics production are three types –**

(i) **Eubacterials** – Most of this type of antibiotic is obtained from **Bacillus spp** 70%. Bacillus subtilis produced more than 60 Antibiotics and from **Pseudomonas species** 30%.
(ii) Actinomycetales [Ramified] – Streptomyces, Micromonospora and streptosporangium. From single species **Streptomyces griseus** more than 40 antibiotics have been obtained.
(iii) Fungi – Penicillium.

4. STEROIDS
They are complex crystallisable lipids having a tetracyclic hydrocarbon core (one 5-carbon and three 6 carbon rings) and a long side chain. They are constituents of hormones and some important biochemicals like cholesterol, progesterone, oestrogen, testosterone, corticosterone, and cortisone. Compounds of steroids are found in both animals and plants. The main important steroid which is found in animals i.e. **Cholestrol**. It is the main constituent of animal cell membrane and main point for the initiation of steroid in hormone inside the body.
Steroids are used medicinally in correcting hormonal imbalance, Anabolic stimulants, Birth control pills, anti-fertility drugs, Anti-inflammatories, relieving pain and suppressing immune responses various steroids differ from one another in radicals like –OH = O, $-CO-CH_3$, $-COCH_2OH$.
Murray and **Peterson** (1950) found that **Rhizopus stolonifer** could bring about hydroxylation required for steroid synthesis including removal of hydrogen [dehydrogenation] from specific carbons.
Different microorganisms produce different steroids from progesterone like pregnane, cortexolone, Androsterone etc. The commercial conversion of **Cortixolone** to **Predinisolone** which is used as **Antiflammatory drugs,** involves first hydroxylation then dehydrogenation.

ORGANIC ACIDS:

Some organic acids are manufactured by employing fermentation activities of Fungi and others of Bacteria.
For example :-
1. Citric Acid :- It is obtained by the aerobic fermentation of sucrose by the fungus **Aspergillus niger**. This acid is used in medicine, flavoring extracts, food and candies; the manufacture of ink, dyeing. It is also produced by yeast.
2. Acetic acid or Vinegar :- Vinegar production is a two step fermentation process :-
A. First step:- Alcoholic fermentation of a carbohydrate into alcohol by yeast.
B. Second step : Aerobic oxidation of alcohol into acetic acid by the Bacterium **Acetobactoer aceti**.
Vinegar is a French word, meaning - **sour wine**, and was known to man thousands of years ago.

The Vinegar is the product of microbial fermentation, was recognized by **Kutzing** in 1837. In 1868 **Pasteur** discovered it to be a result of Biological Activity.
Vinegar is used in various ways in homes. It is used as a condiment and for preserving pickles, canned vegetables and fruits.
Medicinally, it has an important role in promoting digestion and in overcoming constipation.
3. Lactic Acid :– Produced by fermentation of corn starch, Molasses potatoes and whey by **Lactobacillus bulgaris** and **Streptococcus lactis**.
4. Gluconic acid:– Produced from glucose by fungus like Aspergillus, Penicillium and Mucor.
5. Fumaric acid – Produced from Sugar by activity of Rhizopus nigricans [Bread mould]

6. Butyricacid – Obtained by clostidium butylicum.

INTERFERON

First of all Issac and Lindemann observed that immunity due to the formation of special soluble substances, produced by viral infected cells. This small group of protein is named - Interferons.
These are the proteins released by the cells in response to a viral infection which they help to combat. These interferon do not inactivate the virus, but they make the unattacked cells, less susceptible so they are prevented from the attack of virus. They also prevent the viruses from taking over the cellular machinery. Interferon proteins have proved to be effective in treating influenza and hepatitis, but their role in cancer treatment is doubtful. Interferon produced by Charlse Weisman through the E.coli strain produced by Recombinant DNA technology.
There are three major types of interferons :
(1) Interferon-a (INF - a, produced by leucocytes or white blood cells,)
(2) Interferon-b (INF - b, produced by fibroblasts), and
(3) Interferon-g (INF -g produced by stimulated T-lymphocyte cells, hence also called immune interferon.

DEXTRINS

It is a plasma expander having 6-10% solution of dextrins which is given in case of haemorrhage, shock and dehydration and plasma transfusion.
Dextrins are soluble polyglycan for polymers of D-glucose. They are prepared either through partial hydrolysis of starch or partial polymerisation of simple sugars through microorganism Leuconostoc mesenteroides or Enzyme dextran sucrase. The enzyme is more useful as dextran or dextrin of suitable molecular weight can be obtained more easily.

INSULIN

It is a proteinaceous hormone having 51 Amino acids arranged in two polypeptides A and B having 21 and 30 Amino Acids, respectively and joined by S-S disulphide bridges.
Sir Edward sharpy-Shafer (1916) was the first to not that diabetes of some persons was because of failure of some islands of pancreas to produce a substance which he called insulin (Derived from the latin, insula, meaning island).
Banting and best (1921) were the first to isolate insulin from dog's pancreas and used it to cure diabetes in man.
The source of insulin used for curing diabetes these days, is the pancreas of slaughtered cattle and pigs.
Through this insulin is effective in controlling diabetes, it results in certain undesirable effects.
The first genetically engineered insulin obtained by recombinant DNA technique with the help of E-Coli was produced by the American firms, Eli-Lilly on July 5, 1983. It has been given the trade name humulin and has been approved for clinical use.

VACCINES

Production of antibodies against of antigens inside the body is the basis of immunity. Process of innoculation of vaccines is called vaccination. Scientific base of vaccination was established by Louis Pasteur. First vaccine discovered by Edward Jenner for small pox.
A vaccine contains either weakened or even killed-attenuation pathogens [serum suspension with virulence] which have still antigens to induce antibody production. All these vaccines are called First generation vaccines.
They are produced by conventional technique.
Recently new vaccines are produced called second generation vaccines. These are produced by Recombinant DNA technology [Genetic Engineering] e.g., Herpes Virus and Hepatitis-B.
Latest vaccines produced synthetically or synthesized vaccines are called third generation vaccines.

AN IDEAL VACCINE

1. It should not be tumerogenic or toxic or pathogenic, i.e., it should be safe.
2. It should have very low levels of side effects in normal individuals.
3. It should not cause problems in individuals with impaired immune system.
4. It should not spread either within the vaccinated individual or to other individuals (live vaccines.)
5. It should not contaminate the environment.

6. It should be effective in producing long lasting humoral and cellular immunities.
7. The technique of vaccination should be simple.
8. The vaccine should be cheap so that it is generally affordable. So far, such an ideal vaccine has not been developed.

PLANT TISSUE CULTURE

Now a days plant tissue culture technique utilized for the production of important compounds, such as Red dye–is called Shikonin which is obtained from Lithospermum erythrorhizon.
It is utilized for the formation of cosmetic materials.
Note :(i) Antirabies vaccine obtained from culture of chick embryo cells [Rabipur]
(ii) Probiotics: These are dietary nutritional supplements containing micro-organisms (eg. Sporlac tablet containing lactobacillus) Some special Techniques Utilized in Biotechnology – Recombinant
DNA technology – A recombinant DNA molecule is produced by joining together two or more DNA segments usually originating from different organisms. More specifically, a recombinant DNA,molecule is a vector (e.g., a plasmid, phage or virus) into which the desired DNA fragment has been inserted to enable its cloning in an appropriate host.
Recombinant DNA molecules are produced with one of the following three objectives :
(1) To obtain a large number of copies of specific DNA fragments,
(2) To recover large quantities of the protein produced by the concerned gene.
(3) To integrate the gene in question into the chromosome of a target organism where it expresses it self.
This technique developed by Genetic engineering. In this techniques first of all isolation of desired gene from any organisms and its transfer and expression into any organism of choice. They are known as transgenic
micro-organisms. Transgenic micro organisms are produced with view to obtain novel pharmaceutical proteins.
For example – Human Insulin is being produced commercially from Transgenic E.coli strain.
Many valuable recombinant proteins are also being produced, using transgenic animal cells lines and transgenic plants.
At the same time, a number of these proteins of great medicinal value could not be produced on a commercial scale using the non-transgenic cells or organisms.
Proteins produced by transgens are called "recombinant proteins". Such type of recombinant genes are utilized for the formation of different products.
Plants, bacteria, fungi and animals whose genes have been altered by manipulation are called Genetically Modified Organisms (GMO).

Genetically modified crops – A transgenic crop is a crop that contains and expresses a transgene. This crop is known as genetically modified crops or GM crops.

Two unique advantages :-

(i) Any gene (from any organism or a gene synthesised chemically) can be used for transfer, and

(ii) The change in genotype can be precisely controlled since only the transgene is added into the crop genome.For example –Hirudinis a protein that prevents blood clotting. The gene incoding hirudin was chemically synthesized and transferred into Brassica napus. Where hirudin accumulates in seeds. The hirudin is purified and used in medicine.

A soil bacteriumBacillus thuringiensis, producescrystal [Cry]protein. This Cry protein is toxic to Larvae of certain insects. Each Cry protein is toxic to a different group of insects. The gene encoding cry protein is called "cry gene". This Cry protein isolated and transferred into several crops. A crop expressing a cry gene is usually resistant to the group of insects for which the concerned Cry protein is toxic. There are a number of them, for example, the proteins encoded by the genes cryIAc and cryllAb control the cotton bollworms, that of cryIAb controls corn borer.

However, gene symbol italics, e.g., cry. The first letter or the protein symbol, on the other hand, is always capital and the symbol is always written in roman letters, e.g., Cry.

Bt Cotton : 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 . The Bt toxin protein exist as inactive protoxins but once an insect ingest the inactive toxin, it is converted into an active form of toxin due to the alkaline pH of the gut which solubilises 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.

Transgenic variety of Tomato – Flavr Savr due to the inhibition of polygalacturonase enzyme which degrades pectin. So that tomato variety remains fresh and retain flavour much longer.

GM crops are already in cultivation in U.S.A., Europe and several other countries. In India, some insect resistant cotton varieties expressing cry genes have reached the farmers, fields. It has been argued that transgenic crops may be harmful to the environment. The three points. Firstly, the transgene may be transferred through pollen from these crops to their wild relatives.

Secondary, the transgenic crops may themselves become persistent weeds. Thirdly, GM crops may pollute the environment.

Transgenic Animals & Plants

APPLICATIONS OF TRANSGENIC PLANTS:

1. They have proved to be extremely **valuable tools** in studies on plant molecular biology, regulation of gene action, identification of regulatory promontory sequences etc.
2. Specific genes have been transferred into plants to **improve their agronomic** and other features.
3. Genes for **resistance to various biotic stresses** have been engineered to generate transgenic plants resistant to **insects viruses etc.**
4. Several gene transfers have been aimed at improving the **produce quality e.g., protein or lipid** quality etc. of transgenic plants ;
5. Transgenic plants are aimed to produce novel biochemicals like interferon, insulin, immunoglobulins etc.,
6. Transgenic plants have been produced that express a gene encoding an antigenic protein from a pathogen.
7. Made crops more tolerant to abiotic stresses (cold, drought, salt, heat).
8. Reduced reliance on chemical pesticides (pest-resistant crops).
9. Helped to reduce post harvest losses.
10. Increased efficiency of mineral usage by plants (this prevents early exhaustion of fertility of soil).
11. Enhanced nutritional value of food e.g., Vitamin 'A' enriched rice.

Genetic modified food– The food is prepared from genetically modified crop[transgenic] is called genetically modified food or **G.M.Food**.
GM food differs from the food prepared from the produce of conventionally developed varieties mainly in the following aspects .**Firstly**, it contains **the protein** produced by the trangene in question, e.g., Cry protein in the case of insect resistant varieties. Secondly,**it contains the enzyme** produced by the **antibiotic resistance gene** that was used **during gene transfer by genetic engineering**. Finally, it contains the antibiotic resistance gene itself.
GM foods could lead to the following problems when they are consumed. Firstly, the **transgene product** may **toxicity and or produce allergies**. Secondly, the enzyme produced by the antibiotic resistance gene could cause allergies, since it is a foreign protein.**Finally**, the bacteria present in the alimentary canal of the humans could take up the antibiotic resistance gene that is present in the GM food.

Biochemical Production Plants are the chief source carbohydrates, e.g., starch, sugar etc., lipids, proteins, and a variety of unique biochemicals. Transgenes have been shown to introduce novel branches in the biosynthetic pathways of plants and, thereby, to generate valuable products or to produce new, valuable proteins virtually all the cases are promising and in developmental stages, **except for the**

thrombin inhibitor protein hirudin, which is the first commercial example - Hirudin.

TRANSGENIC ANIMALS:

Animals that have had their DNA manipulated to possess and express an extra (foreign) gene are known as **transgenic animals.**Transgenic rats, rabbits, pigs, sheep, cows and fish have been produced, although over 95 percent of all existing transgenic animals are mice.

(i) **Normal physiology and development :** Transgenic animals can be specifically designed to allow the study of how genes are regulated, and how they affect the normal functions of the body and its development, e.g., study of complex factors involved in growth such as **insulin-like growth factor**. By introducing genes from other species that alter the formation of this factor and studying the biological effects that result, information is obtained about the biological role of the factor in the body.

(ii) **Study of disease :** Many transgenic animals are designed to increase our understanding of how genes contribute to the development of disease. These are specially made to serve as models for human diseases so that investigation of new treatments for diseases is made possible. Today transgenic models exist for many human diseases such as cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's
(iii) **Biological products :** Medicines required to treat certain human diseases can contain biological products, but such products are often expensive to make. Transgenic animals that produce useful biological products can be created by the introduction of the portion of DNA (or genes) which codes for a particular product such as human protein (a-1-antitrypsin) used to treat emphysema. Similar attempts are being made for treatment of phenylketonuria (PKU) and cystic fibrosis. In 1997, the first transgenic cow, Rosie, produced human protein-enriched milk (2.4 grams per litre). The milk contained the human alpha-lactalbumin and was nutritionally a more balanced product for human babies that natural cow-milk.
(iv) **Vaccine safety :** Transgenic mice are being developed for use in testing the safety of vaccines before they are used on humans. Transgenic mice are being used to test the safety of the polio vaccine. If successful and found to be reliable, they could replace the use of monkeys to test the safety of batches of the vaccine.
(v) **Chemical safety testing :** This is known as toxicity/safety testing. The procedure is the same as that used for testing toxicity of drugs. Transgenic animals are made that carry genes which make them more sensitive to toxic substances than non-transgenic animals. They are then exposed to the toxic substances and the effects studied. Toxicity testing in such animals will allow us to obtain results in less time.

Sustainable Agriculture – Sustainable agriculture primarily use renewable resources, would cause **minimum pollution and maintain the optimum** yield level. Any development that reduce the use of non renewable resources and level of pollution, would enhance the sustainability of agriculture. Biotechnology play significant role in sustainable agriculture by the use of **biofertilizer**, **biopesticides, disease and insect resistant varieties etc.** **Biopatent** – A patent is a right granted by a government to an **inventor** to prevent others from commercial use of his invention. A patent is granted for – (A) An invention [including product]
(B) An improvement in an earlier invention
(C) The process of generating products and
(D) A concept or design.
These products are called biopatents because they are granted for biological entities.
Biopatents are awarded for the following :
(i) strains of microorganisms,
(ii) cell lines,
(iii) genetically modified strains of plants and animals,
(iv) DNA sequences,
(v) the proteins encoded by DNA sequences,
(vi) various biotechnological procedures,
(vii) production processes,
(viii) products, and
(ix) product applications.
For example, one patent covers**'all transgenic plants of Brassica family'**. Such broad patents are considered morally unacceptable and fundamentally inequitable.

Biopiracy – Many organisations and multinational companies exploit or patent bilogical resources or bioresources of other nations without proper authorisation from the countries concerned is known as biopiracy.
The industrialised nations are rich in technology and financial resources but poor in biodiversity and traditional knowledge related to the utilisation of the bioresources. In contrast, developing nations are poor in technology and financial resources, but are rich in biodiversity and traditional knowledge related to bioresources.

Biological resources or bio-resources include all those organisms that can be used to derive commercial benefits. Traditional knowledge related to bio-resources is the knowledge developed by various communities over long periods of history, regarding the utilisation of the bio-resources, e.g., use of herbs, etc, as drugs. Institutions and companies of industrialised nations are collecting and exploiting the bio-resources, as follows.

(i) The are collecting and patenting the genetic resources themselves. For example, a patent granted in U.S.A. covers the entire 'basmati' rice germplasm indigenous to our country.
(ii) The bio-resources are being analysed for identification of valuable biomolecules. A biomolecule is a compound produced by a living organism. The biomolecules are then patented and used for commercial activities.
(iii) Useful genes are isolated from the bio-resources and patented. These genes are then used to generate commercial products.
A west African plant Pentadiplandra brazzeana produces a protein called Brazzein which is approximately 2000 times more sweeter than sugar. It is proposed to transfer the Brazzein gene into maize and express it in maize Kernels.
But the protein Brazzein was patented in U.S.A. Subsequently, the gene encoding brazzein was isolated and patented in U.S.A.
Bio-war – Bio-war or biological war is the use of biological weapons against humans or their crops and animals.
Bio-weapons are a device that carries and delivers to the target organism in the form of pathological biological agents or toxins called "bio-weapon agent".
Bio-weapon agent is kept in a suitable container so that it remains active and virulent during delivery. This container could be delivered in various ways, including missiles and aircraft. The use of biological agents in war may date back to 5th century B.C.
Some of the potential pathogen for bio-weapons are those that causes Anthrax, Small pox and Botulinum toxin. These bio weapons sent either through letters (envelopes) as powder or in the form of spray.
The possible defence against bio-weapons include the use of respirator or gas mask, vaccination, administration of appropriate antibiotics, and decontamination. In addition, sensitive detection systems should be developed to control and minimise damage.

Benefits of Biological Weapons over Conventional Weapons -.

i) Biological weapons or agents for biological warfare are not costly and can be produced with ease. ii). Detection is almost impossible. iii). Use is likely to increase. iv). They are more dangerous than conventional weapons.

Potential Biological Weapon Agents -.

i Anthrax → Bacillus anthracis. ii. Botulinum toxin (Botulin) → Clostridium botulinum. iii. Plague → Yersinia pestis. iv. Viral Encephalitides → Alpha virus.
Ethics includes a set of standards by which a community regulates its behaviour and decides as to which activity is legitimate and which is not. Therefore bioethics may be viewed as a set of standards that may be issued to regulate our activities in relation to the biological world.
Therefore, the Indian Government has set up organisations such as GEAC (Genetic Engineering Approval Committee), which will make decisions regarding the validity of GM research and the safety of introducing GM organisms for public services.
The major bioethical concerns pertaining to biotechnology are summarised below.
(i) Use of animals in biotechnology causes great suffering to them.
(ii) When animals are used for production of pharmaceutical proteins, they are virtually reduced to the status of a 'factory'.
(iii) Introduction of a transgene from one species into another species violates the 'integrity of species'.
(iv) Transfer of human genes into animals (and vice-versa) dilutes the concept of 'humanness'.
(v) Biotechnology is disrespectful to living beings, and only exploits them for the benefit of human beings.
(vi) Biotechnology may pose unforeseen risks to the environment, including risk to biodiversity.
Bioethics in Plant Genetic Engineering The GM crops are fast becoming a part of agriculture throughout the world because of their contribution to the increased crop productivity and to global food, feed and fiber security, besides their use in health-care and industry. However, the constraints associated with public acceptance of transgenic crops continue to be important challenges facing the global community.
The following are the major concerns about GM crops and GM food :

- The safety of GM food for human and animal consumption (e.g. GM food may cause allergenicity).
- The effect of GM crops on biodiversity and environment.
- The effect of GM crops on non-target and beneficial insects/microbes.
- Transgenes may escape through pollen to related plant species (gene pollution) and may lead to the development of supper weeds.
- The GM crops may change the fundamental vegetable nature of plants as the genes from animals (e.g. fish or mouse) are being introduced into crop plants.
- The antibiotic resistance marker genes used to produce transgenic crops may horizontally transfer into microbes and thus exacerbate problem of antibiotic resistance in human and animal pathogens (i.e. transgenes may move from plants to gut microflora of humans and animals).

- The GM crops may lead to the change in the evolutionary pattern.

Bio-pesticides: Bio-pesticides are those biological agents that are used for control of weeds, insects and pathogens. The micro-organisms used as bio-pesticides include viruses, bacteria, fungi, protozoa and mites.

One example is the soil bacterium, Bacillus thuringiensis. Spores of this bacterium produce the insecticidal Cry protein. Therefore, spores of this bacterium kill larvae of certain insects. The commercial preparations of B. thuringiensis contain a mixture of spores, Cry protein and an inert carrier. This bacterium was the first bio-pesticide to be used on a commercial scale in the world.

The very familiar beetle with red and black markings-the Ladybird, and Dragonflies are useful to get rid of aphids and mosquitoes, respectively. An example of microbial bio-control agents that can be introduced in order to control butterfly caterpillars is the bacteria Bacillus thuringiensis (often written as Bt). These are available in sachets as dried spores which are mixed with water and sprayed onto vulnerable plants such as brassicas and fruit trees, where these are eaten by the insect larvae. In the gut of the larvae, the toxin is released and the larvae get killed. The bacterial disease will kill the caterpillars, but leave other insects unharmed.

Fungal pathogens are attractive bio-control agents for weed control in view of their host specificity and ease in production and inoculation in the field where, A biological control being developed for use in the treatment of plant disease is the fungus Trichoderma.

Trichoderma species are free-living fungi that are very common in the root ecosystems. They are effective bio-control agents of several plant pathogens.

Baculoviruses are pathogens that attack insects and other arthropods. The majority of baculoviruses used as biological control agents are in the genus Nucleopolyhedrovirus. These viruses are excellent candidates for species-specific, narrow spectrum insecticidal application. They have been shown to have no negative impacts on plants, mammals, birds, fish or even on non-target insects. This is especially desirable when beneficial insects are being conserved to aid in an overall integrated pest management (IPM) programme, or when an ecologically sensitive area is being treated.

Between bio-pesticides and chemical pesticides

	Biopesticides	**Chemical pesticides**
1.	These do not harm nontarget species.	Nontarget species are also harmed.
2.	They do not pollute the environment.	Cause pollution by chemical farming; sometimes serious.
3.	No harmful residues remain in food, fodder and fibers	Harmful residues may often remain in food,fodder and fibers.
4.	Relatively Cheaper.	Relatively costlier

5.	Insects are expected not to develop resistance to Bio-pesticides.	Insects may become resistant, e.g., Heliothis Has become resistant to most insecticides.
6.	Since they are highly specific, correct identification of the pest is essential.	It is often not critical.
7.	High specificity may often make the use of two or more bio-pesticides necessary.	often not required.
8.	Performance may be variable due to the influence of biotic and abiotic factors of the environment.	This is not often the case.

Biofertilisers: Bio-fertilisers are organisms that enrich the nutrient quality (N,P) of the soil. The main sources of bio-fertilisers are bacteria, fungi and cyanobacteria.
Bacteria Rhizobium (symbiotic bacteria) fix atmospheric nitrogen into organic forms, which is used by the plant as nutrient.
Other bacteria like Azospirillum and c Azotobacter can fix atmospheric nitrogen while free-living in the soil thus enriching the nitrogen content of the soil.
Fungi are also known to form symbiotic associations with plants (mycorrhiza).
Many members of the genus Glomus form mycorrhiza.
Fungal symbiont in these associations absorbs phosphorus from soil and passes it to the plant. Also provide resistance to root-borne pathogens, tolerance to salinity and drought and cause an overall increase in plant growth and development.
Cyanobacteria are autotropic microbes widely distributed in aquatic and terrestrial environments many of which can fix atmospheric nitrogen. (e.g. Anabaena, Nostoc, Oscillatoria, etc.) In paddy fields, cyanobacteria serve as an important bio-fertiliser.
Blue green algae also add organic matter to the soil and increase its fertility.
Bio-fertilisers are a low-cost input and they do not pollute the environment. They are use to replenish soil nutrients. They also reduce the dependence on chemical fertilisers and also help to use organic farming.

Ethical Issues

Ethical Issues

The manipulation of living organisms by the human race cannot go on any further, without regulation. Some ethical standards are required to evaluate the morality of all human activities that might help or harm living organisms. Going beyond the morality of such issues, the biological significance of such things is also important. Genetic modification of organisms can have unpredictable results when such organisms are introduced into the ecosystem. Therefore, the Indian Government has set up organisations such as GEAC (Genetic Engineering Approval Committee), which will make decisions regarding the validity of GM research and the safety of introducing GM-organisms for public services. The modification/usage of living organisms for public services (as food and medicine sources, for example) has also created problems with patents granted for the same.

There is growing public anger that certain companies are being granted patents for products and technologies that make use of the genetic materials, plants and other biological resources that have long been identified, developed and used by farmers and indigenous people of a specific region/country.

Rice is an important food grain, the presence of which goes back thousands of years in Asia's agricultural history. There are an estimated 200,000 varieties of rice in India alone. The diversity of rice in India is one of the richest in the world. Basmati rice is distinct for its unique aroma and flavour and 27 documented varieties of Basmati are grown in India. There is a reference to Basmati in ancient texts, folklore and poetry, as it has been grown for centuries. In 1997, an American company got patent rights on Basmati rice through the US Patent and Trademark Office. This allowed the company to sell a 'new' variety of Basmati, in the US and abroad.

This 'new' variety of Basmati had actually been derived from Indian farmer's varieties. Indian Basmati was crossed with semi-dwarf varieties and claimed as an invention or a novelty. The patent extends to functional equivalents, implying that other people selling Basmati rice could be restricted by the patent. Several attempts have also been made to patent uses, products and processes based on Indian traditional herbal medicines, e.g., turmeric neem. If we are not vigilant and we do not immediately counter these patent applications, other countries/individuals may encash on our rich legacy and we may not be able to do anything about it.

Biopiracy is the term used to refer to the use of bio-resources by multinational companies and other organisations without proper authorisation from the countries and people concerned without compensatory payment. Most of the industrialised nations are rich financially but poor in biodiversity and traditional knowledge. In contrast the developing and the underdeveloped world is rich in biodiversity and traditional knowledge relative to bio-resources.

Traditional knowledge related to bio-resources can be exploited to develop modern applications and can also be used to save time, effort and expenditure during their commercialization. There has been growing realisation of the injustice, inadequate compensation and benefit sharing between developed and developing countries. Therefore, some nations are developing laws to prevent such unauthorised exploitation of their bio-resources and traditional knowledge. The Indian Parliament has recently cleared the second amendment of the Indian Patents Bill, that takes such issues into consideration, including patent terms emergency provisions and research and development initiative.

Single Cell Protein & Bioenergy

SINGLE CELL PROTEIN:

It is a microbial biomass. This biomass is obtained from both mono and multicellular microorganism.
Single cell protein can be produced using algae, fungi, yeast and bacteria.
Commercial production of S.C.P. is mostly based on yeasts and some other fungi e.g. **fusarium graminearum**.
(i) SCP may be used directly as human food supplement, or else
(ii) It may be used in animal feed to at least partially replace the currently used protein-rich soyabean meal and fish proteins, and even cereals, which can be diverted for human consumption

ADVANTAGES OF SCP

The SCP processes and products offer several advantages as listed below:
1. The SCP is rich in high quality protein and is rather poor in fats, which is rather desirable.
2. They can be produced all the year round and are not dependent of the climate (except the algal processes).
3. The microbes are very fast growing and produce large quantities of SCP from relatively small area of land.
4. They use low cost substrates and, in some cases, such substrates which are being wasted and causing pollution to the environment.
5. When the substrate used for SCP process is a source of pollution, SCP productioe helps reduce pollution.
6. Strains having high biomass yields and a desirable amino acid composition can be easily selected or ptoduced by genetic enginnering.
7. Some SCPs are good sources of vitamins, particularly B-group of vitamins, as well, e.g., yeasts and mushooms.
8. Mushrooms are considered as delicacy in the human diet.
9. At present,SCP appears to be the only feasible approach to bridge the gap between requirement and supply of proteins.

Production of SCP reguire carbon source and other nitrogen, phosphorus and other nutrients needed to support optimal growth of the selected microorganism. SCP process are highly aerobic (except those using algae).
Therefore aeration must be provided.

BIOENERGY

INTRODUCTION:

Only about 0.2 percent of the solar energy that reaches the earth's surface is converted into biomass. Yet this energy trapped annually in the biomass is about ten times the non-biomass energy from other sources (conventional) used by the people the world over. "Bioenergy is the energy obtained from biological sources", broadaly classified into **Animal energy**and**Biofules.** Coal, petroleum and natural gas are also of biological origin, but are classified as fossil fuels. Bioenergy is widely used in the developing countries especially in the rural areas.

ANIMAL ENERGY:

Animal energy is available in two forms- **Human Muscle Power** (HMP) and **Draught** (pronounced draft) **Animal Power** (DAP). HMP is widely used by women in their domestic chores and by marginal farmers, artisans and non-agricultural labourers HMP forms a sizable part of the energy utilised. It is approximately equivalent to **one-fifth** of the total electricity generated per year in India.

BIOFULES:

Biofules or fules of biological origin have been used by man since the discovery fo fire. In spite of the improved methods of obtaining energy, biofules continue to be a major resource. Biofules are **renewable**, and if used properly and efficiently they can help overcome energy problems of developing countries. With improvements in technology it is becomeing possible to substantially replace fossil fules by biofules. Biomass can be used to generate producer gas to run irrigation pumps, to obtain alcohol, to replace petrol, to generate biogas for cooking and lighting, or merely to generate electricity.
Biofules are obtained from wood and agricultural, agro-industrial and animal wastes, and from plants that produce alcohol, oil and petroleum.

WOOD:

Wood has been used by man from the time he discovered fire. Over half the global population still depends on wood for cooking and heating. Wood is also used as fuel in many industries. The consumption of fuelwood in the world was estimated at 1.7 billion m3 in 1984. Two-thirds of this was utilised by the developing countries in Africa and Asia.

Such massive use of fuelwood has resulted in extensive deforestation and consequent environmental degradation.

A major quantity of wood is used as firewood. The principal advantages of firewood are :

1. It is a widely distributed source of renewable energy.
2. It can be harvested by unskilled labour, using simple equipment
3. When perfectly dry, 99 percent of it is combustible.
4. It is a fuel which produces flame and is well-adapted to heating large surfaces.
5. As all wood is composed essentially of the same substances, wood from most species can be used as fuel.

Plantation of trees for fire wood is called**energy plantation**.

What determines the value of particular wood as fuel.

There are hundred types of wood in a vast country like India. Of these only a few are selected as suitable fuel.

Good firewool must

(i) be highly combustible,
(ii) have a high calorific value,
(iii) be easy to dry,
(iv) not split when ignited,
(v) be non-resinous and non-smoky and
(vi) be free from offensive odours.

Generally speaking hardwood (dicotyledonous) is better than soft wood (gymnospermous) as fuel. The former produces uniform heat over a long period of time. Soft wood burns rapidly to produce intense heat but only for a short period.

Cow Dung Cakes :-In rural areas of developing countries, it is a common practice to use animal dung for making dung cakes which are used for fuel. Thus a potential fertiliser of the agricultural fields is wasted in Burning.

The dung Can be put to a better use if it is used to generate Bio gas (Gobar Gas) and side by side a stabilised residue to serve as the fertiliser.

50 Kg of fresh Buffalo-dung

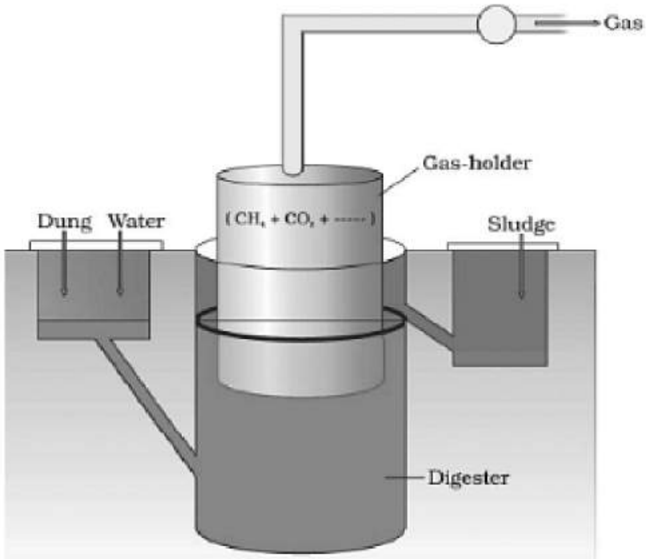
Conventional Use	Biogas Production
10 Kg of dung cake (dry)	$2m^3$ of biogas
30, 000 K.cal (gross energy)	9000 K Cal (gross energy)
10% efficiency in burning in chulhas	30% efficiency in burners
Usefull energy (3000 K.cal) + AIR Pollution	Useful energy (2700 K.Cal) + No pollution + Fertilizers sufficient for one acre of land for two crops / year.

The energy yield of Biogas is lower than that of dung cakes but the efficiency of Biogas burners is very high.

Thus over all result indicates that production of biogas is more cost effective.

The biogas plant consists of a concrete tank (10-15 feet deep) in which bio-wastes are collected and a slurry of during is fed. A floating cover is placed over the slurry, which keeps on rising as the gas is produced in the tank due to the microbial activity. The biogas plan has an outlet, which is connected to a pipe to supply biogas to nearby houses. The spent slurry is removed through another outlet and may be used as fertiliser. Cattle dung is available in large quantities in rural areas where cattle are used for a variety of purposes. So biogas plants are more after build in rural areas. The biogas thus produced is used for cooking and lighting. The technology of biogas production was developed in India mainly due to the efforts of Indian Agricultural Research Institute (IARI) and Khadi and Village Industries Commission (KVIC)

"Gobar Gas" or (Bio Gas)



The organic wastes from the farmhouse cow dung, wastes, urine, faeces etc. can be used economically for producing of Gobar gas (Bio gas). It consists of methane (50-70%), CO2(30-40%) and traces of hydrogen, nitrogen and hydrogen sulphide.
Biogas produced by anaerobic fermentation of waste biomass.
Anaerobic fermentation of waste biomass can be visualised in three stages :-
1. The facultative anaerobic microbes degrade the complex polymers to simple monomers by enzymatic action.
The Polymers like cellulose, hemicellulose, proteins and lipids get degraded into monomers but lignins and inorganic salts are left as residue because they do not degraded.
2. In second stage, monomers are converted in to organic acids by microbial action under partially aerobic conditions which are finally converted to acetic acid.
3. In third stage acetic acid is oxidised in to methane by the acitivity of anaerobic methanogenic bacteria. These bacteria are commonly found in the anaerobic sludge during sewage treatment. These bacteria are also present in the rumen (a part of stomach) of cattle. A lot of cellulosic material present in the food of cattle is also present in the rumen. In rumen, these bacteria help in the breakdown of cellulose and play an important role in the nutrition of cattle. In this whole process digestion ofcellulosetakes place at very slow rate so that it is the "rate limiting factor in biogas production".

Advantage:

1. Biogas can be easily stored to provide more efficient source of energy.

2. It can be used for various purposes in addition to its use for cooking.
3. One bye product of this process is a stabilised residue which serves as a good fertilizer.
4. It reduces the overgrowth of faecal pathogens because of non availability of exposed waste. Thus it is significant in improving sanitation.
5. It also reduces the chances of spreading of pathogens in the field condition, minimising the incidence of diseases in a crop year after year.
6. Its calorific value is about 23-28 MJ/m3.
Plants as Sources of hydrocarbons for Producing Petroleum Melvin Calvin has identified certain plants which produce hydrocarbons. The plants of Euphorbiaceae, Asclepiadaceae and Apocyanaceae produce latex a milky secretion which contains hydrocarbons.
The liquid hydrocarbon of the latex can serve as the liquid fuel which can replace the fuel requirement of automobiles either mixed with petrol or as entire fuel. Plantation of such type of plants are called Petro Plantation. This is the such type of method in which source is available only through the demand.

Alcohol as fuel:

1. Ethanol (C_2H_5OH) can be partly or wholly substituted for petrol in automobiles after suitable modifications in Engines.
2. Raising of crops like Sugarbeet, Potato, Maize, Sugarcane, Tapioca and Molasses for producing ethanol is called energy croping.
3. Alcohol has been successfully used as motor fuel in Brazil and it is the first leading country in the world. "Ethanol from starch and lignocellulose"
Ethanol or Ethyl Alcohol obtained from starch sources.
Ex. Potato, Molasses, waste sulphite liquor and wood sugars (lignocellulose) Method :- The starch is hydrolysed into sugars which is then fermented in to alcohol.

$$\underset{\text{glucose}}{C_6H_{12}O_6} \xrightarrow[\text{Zymase}]{\text{yeast}} \underset{\text{Ethanol}}{2C_2H_5OH} + \underset{\text{Carbondioxide}}{2CO_2}$$

Yeast, Enzymes & Yogurt

BIOTECHNOLOGY

DEFINITION –

"Biotechnology may be defined as use of micro-organism, animals, or plant cells or their products to generate different products at industrial scale and services useful to human beings."

A powerful industry based on microbes has been developed in recent time. A careful selection of microbial strains, improved method of extraction and purification of the product, have resulted in enormous yields.
The use of living organisms in systems or process for the manufacturer of useful products, It may involve algae, bacteria, fungi, yeast, cells of Higher plants & animals or subsystems of any of these or Isolated components from living matter.

Old biotechnology are based on the natural capabilities of micro organisms. e.g. formation of Citric acid, production of penicillin by Penicillium notatum **New biotechnology is based on Recombinant DNA technology.** e.g. Human gene producing Insulin has been transferred and expressed in bacteria like E.coli.
In,**modern biotechnology**, different types of valuable products are produced with help of microbiology, biochemistry, tissue culture, chemical engineering and genetic engineering, molecular biology and immunology.

MICROBES IN HOUSEHOLD PRODUCTS

A common example is the production of curd from milk. Micro-organisms such as Lactobacillus and others commonly called **lactic acid bacteria (LAB)** grow in milk and convert it to curd. During growth, the LAB produce acids that coagulate and partially digest the milk proteins. A small amount of curd added to the fresh milk as inoculum or starter contain millions of LAB, which at suitable temperatures multiply, thus converting milk to curd, which also improves its nutritional quality by increasing vitamin B12. In our stomach too, the LAB play very beneficial role in checking disease causing microbes.
The dough, which is used for making foods such as dosa and idli is also fermented by bacteria. The puffed-up appearance of dough is due to the production of CO_2 gas. Similarly the dough, which is used for making bread, is fermented using baker's yeast (Saccharomyces cerevisiae). A number of traditional drinks (e.g. ''Todi' prepared from sap of palms) and foods are also made by fermentation by the microbes. microbes are also used to ferment fish, soyabean and bamboo shoots to make foods. Cheese, is one of the oldest food items in which microbes were used. Different varieties of cheese are known by their characteristic textur flavour and taste, the specificity coming from the microbes used. For example, th large holes in 'Swiss cheese' are due to production of a large amount of CO_2 by a bacterium named **Propionibacterium sharmanii.** The 'Roquefort cheese' are ripened by growing a specific fungi on them, which gives them a particular flavour.

YEAST

Louis Pasteur showed in the middle of nineteenth centuary that **beer** and **butter milk** are product of fermentation brought about by "**yeast**". It is a microscopic single celled organism –**Saccharomyces cerevisiae**.
Presently however yeast product for human and animal consumption are produced on commercial scale. "**Alcohol**was the first product of ancient biotechnology"
There are basically two types of yeasts (i) Baker's yeast (ii) Alcohol yeast or Brewer's yeast Baker's yeast generally utilize during the preparation of food materials to increase the taste of food, flavour in food and nutrients in food. It is also utilized as "**leavening agent**".
By the incomplete degradation of complex organic compounds [sucrose] by yeast fermentation, alcohol is formed.

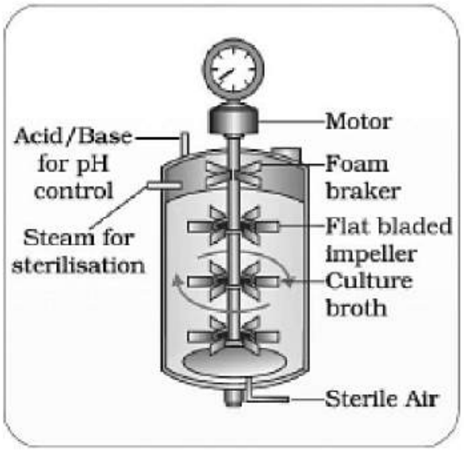
$$\underset{\text{[Sucrose]}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow[\text{invertase}]{\text{yeast}} \underset{\text{[Glucose]}}{C_6H_{12}O_6} + \underset{\text{[Fructose]}}{C_6H_{12}O_6}$$

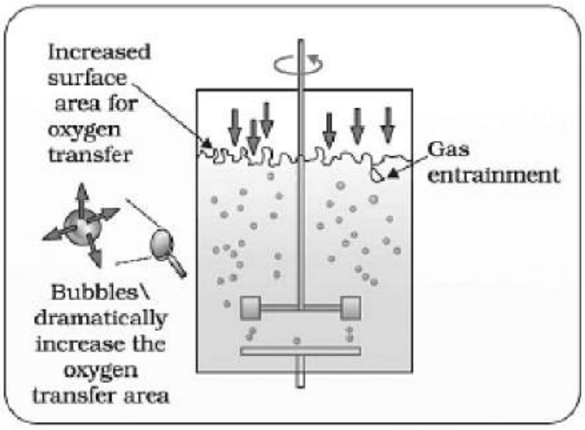
$$C_6H_{12}O_6 \xrightarrow[\text{zymase}]{\text{yeast}} \underset{\text{[Ethyl alcohol]}}{2C_2H_5OH} + 2CO_2$$

Some other common products of yeast fermentation are –
[i] Beer – It is produced from **Hordeum Vulgare**[Barely] malt and alcohol content is 4-8%
[ii] Wine – Produced from **grapes**, alcohol content is 10-20%.
[iii] Brandy – Produced by distilation of wine and alcohol content is 43-57%
[iv] Gin – Produced from **European Rye-Scale cereal**.
[v] Rum – Produced from **Molasses** of Sugarcane and alcohol contents is 40%
Note – Another yeast which supplies nutritional rich food for Man and animals is **Torulopsis utilis**.

Industrial utilization of biotechnology involve three steps –
[i] Laboratory scale process
[ii] Pilot plant scale
[iii] Manufacturing unit The development from laboratory scale to manufacturing unit is "Scaling up to industrial production"

[i] Laboratory Scale - In this process for the production of desirable product, proper micro organism searched and then suitable strain is selected and multiplied. Proper medium also find out on which selected strain, produce best and more amount of product.
Many number of experiments performed in lab for the analysis and selection of strains and medium. All the equipment are utilized in lab i.e., glass apparatus. All the parameters of the process worked out and precaution are also not down for the smooth running of process such as – proper sterlization of nutrient and microbesstrain, required - pH, suitable aerator, disposal of CO_2 if evovled, temperature, by product or product inhibition or stimulation, time of optimum production, separation of product and its purification etc. Ultimately, the laboratory scale process finalized and transfer at pilot plant scale.
[ii] Pilot plant Scale – It is the intermediate stage where working of laboratory scale process is tested. At this stage **cost** and **quality** of product throughly checked. Glass apparatus are replaced by stainless steel equipment/containers is called "**bio reactor**".
To produce in large quantities, the development of **bioreactors.** where large volumes (100-1000 litres) of culture can be processed, was required. Thus, bioreactors can be thought of as vessels in which raw materials are biologically converted into specific products, individual enzymes, etc., useing microbial plant, animal or human cells. A bioreactor provides the optimal conditions for achieving the desired product by providing optimum growth conditions (temperature, pH, substrate, salts, vitamins, oxygen).
The most commonly used bioreacters are of stirring type





A stirred-tank reactor is usually cylindrical or with a curved base to facilitate the mixing of the reactor contents.
The stirrer faciliates even mixing and oxygen availability throughout the bioreactor. Alternatively air can be bubbled through the reactor. The bioreactor has an agitator system, an oxygen delivery system and a foam control system, a temperature control system, pH control system and sampling parts so that small volumes of the culture can be withdrawn periodically.
Micro organisms can be grown in bioreactors in two ways:

(a) **Support growth system** – In this method microorganisms are growing as a thin layer or film in the **solid medium.**

(b) **Suspended growth system** – By suspending cells or mycelia in the **liquid** medium is called suspended growth system.
[iii] Manufacturing unit – During the designing of bioreactor for the process often very large size so that it accomodate huge amount of medium.

Downstream Processing – After completion of the biosynthetic stage, the product has to be subjected through a series of processes before it is ready for marketing as a finished product. The processes include separation and purification, which are collectively referred to as downstream processing. The product has to be formulated with suitable preservatives.
Such formulation has to undergo through clinical trials as in case of drugs. Strict quality control testing for each product is also required. The downstream processing quality control testing vary from product to product.
Some important biotechnological products which are produced with the help of organisms as follows –

1. ENZYME

Total known enzymes 2,200 and only 1–1.5% are used
(i) **Rennet** – Manufacturing "**Cheese**"
Old days cheese had been prepared either using the layer of stomach of Goat or Sheep OR the sap of **Fig. tree,** containing special enzyme–**Ficin**. In 1874 a Danish Chemist – **Christian Hansen** extracted pure rennet enzyme from **Calf's stomach** for industrial production of cheese. First of all diastase enzyme was identify by payen and persoz (1933) Cheese is mainly two different types.
I. Unripened cheese – Ripened from out side–soft
II. Ripened cheese– It is hard and ripened externally as well as internally.
Manufacturing cheese involve following steps.
(i) Milk is inoculated with starter culture of bacteria – **Streptococcus lactis** or **S.cremoris** and warmed at 380C. If higher temperature [500C or more] then **Sthermophilus** combined with **Lactobaccilus lactis**, **Lbulgaricus** or **Lhelveticus**.
(ii) When a certain **acidity** reached in milk by the activity of species of bacteria then rennet enzyme is added. Curdling of milk occurs within half an hour to one hour.
(iii) The curd is removed and liquid separates out which is called **whey** [contain 93% water and 5% Lactose].
Lactose of whey is used for the manufacture of **Lactic acid** – First fermented acid.
If the cheese is used at this stage is called**cottage cheese**(unripened stage).
(iv) The salts mixed with cottage cheese and put into the frames and pressed so as to allow removal of whey.
Salts hastens the removal moisture and prevent the growth of undesriable microbes.
The frames are removed as soon as the cheese has set sufficiently to maintin its shape.
The ripening period varies from 1–16 months but which is very tasty and nutritious.
This is hard and ripened cheese contains about 20-30% fats, 20-35% proteins and small amount of minerals and vitamins. [Cheese which prepared at homes with the

help of lemon juice is called **Raw cheese**] Nearly 400 varieties of cheese available which can be classified into following type –

	Type of Cheese	Micro Organisms used	Reaction
1.	Soft 1. Camembert 2. Limburger	Penicillium camemberti , Brevibacterium ,Streptococcus liquifaciens ,Brevibacterium	Ripend by action of microorganisms on the surface of curd
2.	Semi-hard 1. Roquefort 2. Hue	Penirilliim roqueforti	Combination of surface and interior growths
3.	Hard 1. Swiss 2. Cheddar	Propionibacterium sp Geotrichum	Inoculating the organisms throughout the curd

(ii) Proteases – This enzyme obtained from **Aspergillus orizae** and **Bacillus subtilis, Bacillus licheniformis** and utilized from the formation of **detergents** in detergent industry [For removing proteinous strains on clothes]. The bottlejuices are clarified by the use of pectinases and protease.
(iii) Amylases – It works on starch and used in Beer, Bread and Textiles industries.
(iv) Amylase, Gluco amylase and Gluco isomerase – By the action of all these enzymes **corn** (maize) **starch** transformed into **fructose corn syrup**. This syrup is more seeter than sucrose and used in beverage industry to flavour **soft drinks** and in baking industry to sweeten biscuits and cakes.
(5) Tissue Plasminogen Activator [TPA] or Streptokinase – This enzyme utilized in medicinal field.
Streptokinase produced by the bacterium Streptococcus and modified by genetic engineering is used as a clot buster for removing clots from the blood vessels of patients who have undergone myocardial infraction leading to heart attack.

Uses Of Enzymes:

(1) Detergents (i) **Proteases** (ii) **a -Amylase** (iii) **Cellulases** (iv) **Lipases**

(2) Leather Industry
(3) Wool Industries
(4) Glucose from Cellulose
(5) Food, Dairy, Juice and Beverages Industries
(6) Production Of Glucose Syrup Bioactive molecule, cyclosporinA, that is used as an immunosuppressive agent in organ-transplant patients, is produced by the fungus **Trichoderma polysporum**.
Statins produced by the yeast Monascus purpureus have been commercialised as blood -cholesterol lowering agents. It acts by competitively inhibiting the enzyme responsible for synthesis of cholesterol.

"YOGHURT" [CURDS]

For production of curds or yoghurt pasteurized milk is inoculated with a mixture of**Streptococcus thermophilus** and**Lactobacillus bulgaricus** and its lactose is fermented by keeping it at 400C. The peculiar or characteristic taste and flavour of curds are due to presence of lactic acid and acetaldehyde. Curdling or coagulation of milk is also caused by lactic acid which is formed. In India, curds are not generally commercially produced but in developed countries large scale manufacture of yoghurt is done. In U.S.A. alone about 75 lakh kilogram of yoghurt is manufactured every year.

Cloning

CLONING

Clone is the exact carbon copy or copies produced by a single parent (mother or father) by non-sexual methods and are indentical to their parent genetically and morphologically. Clone is a greek word which means twig (Klon=twig). As all the branches of a tree are similar in morphology and genetical characteristics, in the same way clones are also similar to one another.
Cloning is the process of producing many identical organisms (clone), generally used to produce new plants with similar characteristics. Microbes produce clones through asexual reproduction. In higher animals, clones are produced by nuclear transplantation technique in which the nucleus from a somatic cell is transferred into an unfertilized enucleated egg. The world's most famous sheep 'Dolly' was a clone produced by this method.
Many plant species show vegetative reproduction. In these plants, the clones produced by a twig (detached shoot) are similar in their genotype as well as in phenotype (except environmental variations). Scientists have been much curious to apply this characteristics of plants on animals also to conserve the desired genotypes of some rare animals by making their clones. In higher animal, showing

sexual reproduction, a zygote is formed after fertilization of the egg by speromatozoan. Zygote differs from its parents in genotype. It was revealed by the scientists through several experiments that only the egg and/or zygote has the potential to produce a whole individual from a single cell.J.B. Gurdon (1969) of Oxford University applied this fact while performing an experiment on frog. He destroyed the nucleus of an unfertilized egg of frog by treating with U.V rays and transferred the nucleus of intestinal epithelial cell of tadpole into the egg cell. In this experiment a few of the many transplanted eggs could develop into tadpoles. These developed tadpoles were identical in genotype and phenotype to their parents. This nuclear transplantation technique devised by Gurdon is still being used in cloning paractice in some modified manner. A brief introductory history of cloning is given in table.

In addition to the fact depicted in table attempts are continuously in progress in this field. In December, 2001 (report published in February, 2002) scientists at University, Texas, successfully produced the first cloned domestic pet named as copy cat (C.C). Further, in Aug. 2005 Woo-Sukhwang of South Korea produced the clone of an Afganian hound (Domestic dog used for hunting).

An introductory story of cloning

Year	**Name of the Scientist**	**Brief account of the experiment (s)**	**Result**
1950s	Briggs & King	Nuclear transplantation from embryo to egg in frog.	Tadpoles produced but died before adulthood.
1960s	John B. Gurdon	Nuclear transplantation from cells of skin, liver, kidney into the egg in frog.	Tadpoles produced but died before adulthood.
1970s	Illmense	Nuclear transplantation from embryo to egg in Drosophila	Larvae produced but died before adulthood
1984	Mcgrath & Solter	Nuclear transplantation from embryo to egg in mouse	A few mice born but none lived to adulthood.
1993	Hall & Stillman	Atrificial splitting of an embryo of human into two identical twins	First artificially twinned embryos developed but abnormal
March, 1995	Roslin Instituteteam Scotland	Nuclear transplantation from embryo to egg in sheep	Megan and Moragn sheep born normally

Feb, 1997	Roslin Institute team, Scotland	Nuclear transplantation udder cell to egg in sheep	"Dolly" sheep born normally
March, 1997	Don Wolf and Coworkers, Oregon	Nuclear transplantation from embryo to egg in monkey	Two monkeys "Neti"and "Ditto" born normally
Dec. 1997	Roslin Institute team, Scotland	Nuclear transplantation from embryo to egg in sheep	Molly and Polly sheep born normally
1998	University of Hawai	Nuclear transplantation from adult cell to egg in mice	50 mice born normally
1999	Kato and Coworkers	Nuclear transplantation from adult cell to egg in cow	"George and Charlie" cows born normally
2000	Well and his Associated	Nuclear transplantation from skin cell to egg in cow	Many cows born normally
2001	Kabota	Nuclear transplantation from skin fibroblast culture to egg in cow	Six calves born

An Italian scientist Dr. S. Antenori is trying hard to produce human clone. It has triggred serious discussions and debates at global level, focussing on the medical and ethical issues.

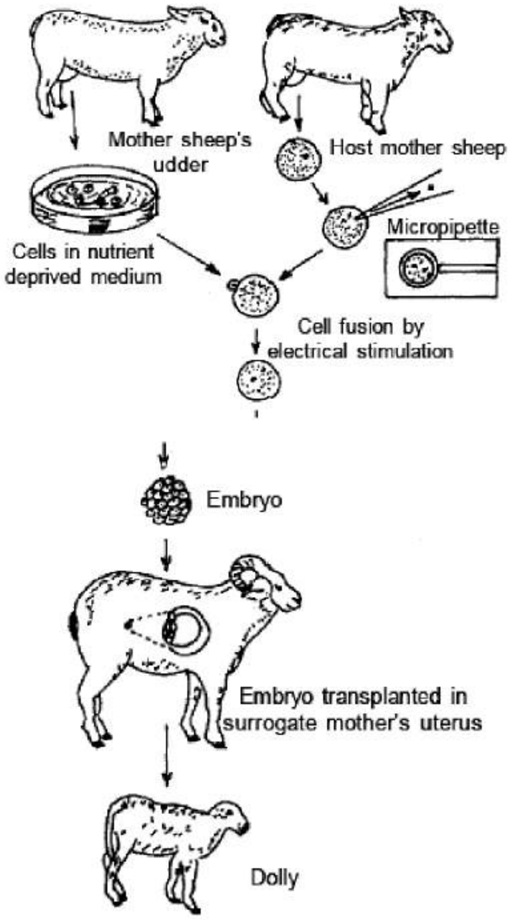
Types of Cloning

Cloning is an extensive technique, which is divided into following types, on the basis of the experimental material used -
(1) Gene cloning (2) Microbial cloning (3) Cell cloning (4) Plant cloning and (5) Animal cloning

Animal cloning

Embryonic cell in animals, are deprived of their totipotency by the time they enter into gastrula stage. So animal cloning is some what more difficult than plant cloning. On the basis of aims and end products animal cloning is of two types
(i) Reproductive and
(ii) Therapeutic.

A clone of the whole animal is prepared in reproductive cloning. The techniques which are used in such cloning include blastomere separation, nuclear transplantation and the Honolulu technique.
This techique can be used to conserve and increase the number of those animals which are threatened to be extinct in the near future.
In nuclear transplantation technique, nucleus is removed from the egg obtained from the female. After then the nucleus of the desired cell (2n) is transferred into the enucleated egg cell which is then allowed to develop into an embryo in suitable conditions. The developing embryo is the clone of the donor cell from which nucleus was obtained for transplantation.
In Honolulu technique 1998-Teruhiko wakayama - cloned mice) there is no fusion of the donor and recipient cells or its nucleus. Instead of nucleus from the donor cell which is in G0 or G1, stage is substituted into enucleated egg cell (culture medium or chemical both is used in place of electric shock to stimulate development).
Therapeutic cloning technique may prove to be very useful in the field of medical science, particularly when there is a need for the transplantation to replace some damaged and diseased organ or tissue by a healthy organ from a suitable donor. In such condition, if the cells from the patient himself are taken and cultured to form desired organ. The organs developed in such manner (organ cloning) will be easily acceptable by the patient, and there will be no possibility of its rejection as often occurs otherwise.
A number of diseases like will be no possibility of its rejection as often occurs otherwise. A number of diseases like parkinsonia, alzheimer, diabetes and diseases related with kidneys will possibly be cured in future by the application of cloning technique. "Dolly" sheep was produced by using nuclear transfer technique by Dr. lan wilmut and his collegues at Roslin Institute of Scotland in 1997.
They used somatic cells from udder (mammary glands) for forming this clone. One udder cell with its nucleus intact was selected because this nucleus carried the mother's genetic information.
Meanwhile, and unfertilized egg cell was taken from a different sheep. Its nucleus was sucked out and an enucleated egg cell was obtained. After then the udder cell nucleus was fused with the enucleated egg cell under electrical stimulation. Now this egg cell had the mother's nucleus. At last the fused egg was implanted into the uterus of surrogate mother, other than the egg donor where it grew into a lamb. Thus the Dolly was born, as a genetically identical copy of its mother



Advantages of Cloning

1. This technique can be used to improve the breeds of live-stock used in agriculture.
2. Cloning is helpful for providing many useful substances and chemicals required for human body, and also in the cloning of such animals which can be used as a source of organs for transplantation purpose in medical practices.
3. British scientists have been successful in obtaining alpha antitrypsin from sheep for curing an incurable disease emphysema and clotting factor- ix for curing haemophilia. It has been possible by the use of genetic engineering.
4. Many incurable diseases which are not curable so far, may be cured effectively in near future by applying the processes relating with cloning and genetic engineering.
5. Cloning is useful to increase the number of individuals of those species which are at the edge of extinction, thus helpful in conservation of biodiversity.

Disputes Related to Cloning

At present, the issue of cloning has been a matter of discussion and disputes among the scientists and the sociologists. Many questions are being raised with regards to the ethical, moral, and social aspects of cloning.

Doubts have also been there about the health and ageing of clones and the misuse of cloning.

Cloning has important role in treatment of serious and non-curable diseases, a view that is favoured by most scientists, however there is no agreement on the issue of human cloning.