12 Biotechnology

Can you recall?

1. What is Biotechnology?
2. How do genetically modified organisms are produced?
3. Which are the benefits of Biotechnology?

You are already aware of what biotechnology is. It is the product of interaction between the biological science and technology. It is infact, an applied branch of biology. The term biotechnology was first used by Karl Ereky in 1919 to describe a process for large scale production of pigs.

12.1 Biotechnology

It is defined by different organizations in different ways. It has been brodly defined as 'the development and utilization of biological forms, products or processes for obtaining maximum benifits to man and other forms of life'. According to OECD (Organization for Economic Cooperation and Development, 1981)- 'It is the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and service to the human welfare'. It uses scientific priciples of microbiology, genetics, biochemistry, chemical engineering, mathematics, statistics, computers, industrial processes, etc. Biological agent means plants and animal cells, microorganisms, enzymes or their products.

History of origin of biotechnology is as old as human civilization. Development of biotechnology in terms of its growth, occurred in two phases viz, Traditional biotechnology and Modern biotechnology.

Traditional biotechnology (old biotechnology) was primarily based on fermentation technology using microorganisms as in the preparation of curd, ghee, soma, vinegar, yogurt (yoghurt), cheese making, wine making, etc. It became an art of kitchen in indian houses. It was more an art than science. Till that time people did not know as to how exactly the process occurs and the organisms causing this process. The contributions made by several chemists, biochemists and microbiologist, over the time, could explain the mechanism of process and also the nature of microorganisms causing the process.

During 1970 a new technique of 'recombinant DNA technology was developed and then established by Stanley Cohen and Herbert Boyer in 1973. This technique has changed the overall outlook, then. The technique permits to change/ modify genetic (heritable) material for getting new specific products. The combination of biology and production technology based on genetic engineering evolved into **modern biotechnology** (new biotechnology).

There are two major features of technology that differentiate modern biotechnology from classical or old biotechnology viz,

i. Capability of science to change the genetic material for getting new specific products through rDNA technology, polymerase chain reaction (PCR), microarrays, cell culture and fusion, and bioprocessing.
ii. Ownership of technology and its socio-political impact.

Now the conventional industries, pharmaceutical industries, agro industries, food industries, etc. are also focussing attention to produce biotechnology- based products.

12.2 Principles and Processes of Biotechnology :

Modern biotechnology is based on two core techniques viz. genetic engineering and chemical engineering.

Genetic engineering deals with alteration of genetic material (DNA and RNA) while chemical engineering deals with maintaining sterile environment for manufacturing variety of useful products including vaccines, antibodies, enzymes, organic acids, vitamins, therapeutics, etc.

Genetic engineering is defined as the manupulation of genetic material towards a desired end and in a directed and predetermined way, using in vitro process. Manipulation of genes involve repairing of the defective genes or replacing of defective genes by healthy genes or normal genes; artificially synthesizing a totally new gene; transfer of genes into a new location or into a new organism; introducing an altogether new gene; manipulation of genes for improvement of living organisms; combining of genes from two organisms, altering the genotype; gene cloning etc. Therefore, the genetic engineering is alternatively called **recombinant DNA technology** or **gene cloning**.

Extra information :

John E. Smith (1996) gave definition of genetic engineering as 'the formation of new combination of heritable material by the insertion of nucleic acid molecule produced by whatever means outside the cells, into any virus, bacterial plasmid or other vector system so as to allow their incorporation into a host organism in which they do not occur naturally but in which they are capable of continued propagation'.

Technique of gene cloning and rDNA technology :

In gene cloning, a gene of known function can be transferred from its normal location into a cell (that of course does not contain it) via a suitable vector. The transferred gene is replicated normally and is handed over to the next progeny.

A. Tools and techniques for gene cloning/ rDNA technology :

Before we venture into a procedure of gene cloning, let us know briefly, the basic requirements for the technique.

I. Different instruments (devices) : Macromolecule such as DNA, RNA, proteins, etc. are synthesized in the living cells which vary in their molecular weight, solubility, presence of charges, absorbance of light, etc. Several techniques are used to isolate and charaterize the macromolecules. The size of different types of molecules varies and therefore their molecular weights also vary. The techniques used on the basis of molecular weight, are gel permeation, ion exchange chromatography, spectroscopy, mass spectrometry, electrophoresis, etc. Electrophoresis is the separation of charged molecules, applying an electric field. It is applied for the separation of DNA, RNA and proteins. DNA being negatively charged, migrates to anode. Small fragments of DNA molecules, move faster and thus separate faster. Use of Agarose gel electrophoresis, PAGE, SDS PAGE are the different methods of electrophoresis.

Polymerase chain reaction (PCR) :

Polymerase chain reaction (PCR) is another device used for gene cloning or gene multiplication in vitro. It is the amplification of gene of interest, through PCR.

In 1985, Kary Mullis made an important discovery (contribution) in the form of an extremely powerful technique called polymerase chain reaction (PCR). PCR can generate a billion copies of the desired segment of DNA or RNA, with high accuracy and specificity, in a matter of few hours. The process of PCR is completely automated and involves automatic thermal cycles for denaturation and renaturation of double stranded DNA.

The device required for PCR is called thermal cycler.

PCR is *in vitro* amplification of a desired DNA segment, which requires : DNA containing the desired segment to be amplified, several molecules of four deoxyribonuclueoside triphosphates (dNTPs), excess of two primer molecules, heat stable DNA polymerase and appropriate quantities of Mg^{++} ions.

Internet my friend

Collect the information from internet about PCR machine.

Mechanism of PCR:

At the start of PCR, the DNA segment, and excess of two primer molecules, four deoxyribonucleosides triphosphates and the thermostable DNA polymerase are mixed together in 'eppendorf tube' and the following operations are performed sequentially (Figure).

Step i : The reaction mixture is heated to a temperature (90–98 ^{o}C) to separate two strands of desired DNA. This is called denaturation.

Step ii : The mixture is allowed to cool (40–60 ^{o}C) that permits pairing of the primer to the complementary sequences in DNA. This step is called annealing.

Step iii : The temperature (70–75 ^{o}C) allows thermostable Taq DNA polymerase to use single-stranded DNA as template and adds nucleotides. This is called primer extension. It takes arround two minutes duration.

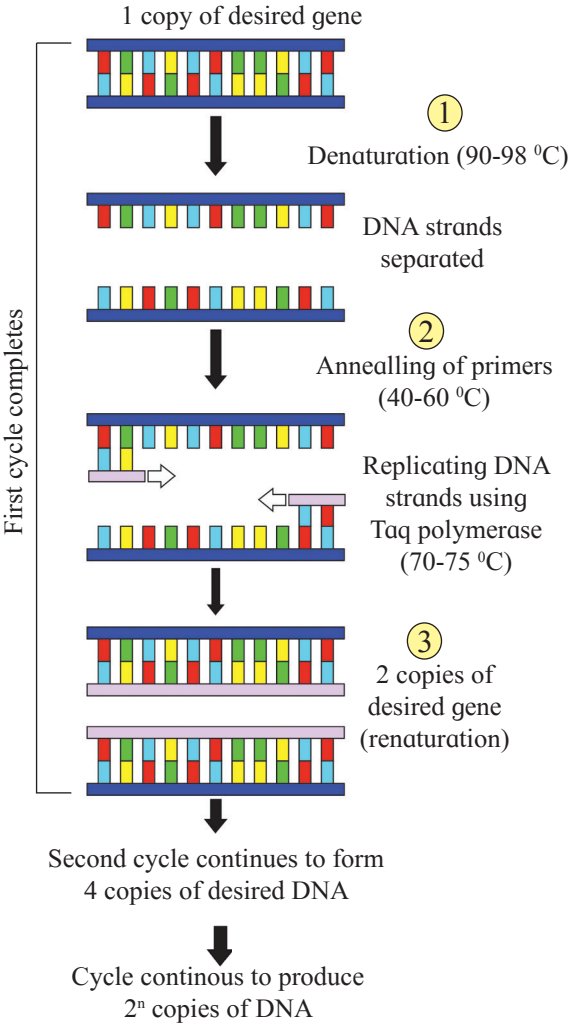

Fig. 12.1 : DNA replication through polymerase chain reaction.

One cycle takes arround 3 to 4 minutes. To begin second cycle, DNA is again heated to convert double stranded DNA into single strands.

In an automatic thermal cycler, the above three steps are automatically repeated 20-30 times. Thus, at the end of 'n' cycles 2^{n} copies of DNA segments, are produed. The machine performs the entire operations automatically and precisely.

Once the desired number of cycles is completed, the amplified DNA segment is purified by gel electrophoresis. After its sequencing, the amplified DNA segment can be inserted into a cloning vector. Desired gene can also be obtained from gene library.

II. Biological tools :

There are three types of biological tools used viz, enzymes, cloning vectors (vehicle DNA) and competent host (cloning organisms) for transformation with recombinant DNA.

A. Enzymes : Different enzymes include Lysozymes, Nucleases such as exonucleases endonucleases, restriction endonucleases, DNA ligases, DNA polymerases, alkaline phosphatases, reverse transcriptases, etc.

i. Restriction enzymes :

Enzymes that cut the phosphodiester bonds of polynucleotide chains are called **nucleases**. These are of two types - exonuclease and endonuclease. Exonucleases cut nucleotides from the ends of DNA strands whereas endonuclease cut DNA from within. During the 1970s, it was found that bacteria contain nucleases that would recognize short nucleotide sequence with duplex DNA and cut.

The phosphodiester back bone at highly specific sites on both strands of duplex, is cut by these enzymes, called **restriction endonucleases (REN)** or simply **restriction enzymes (RE)**. They were given this name because they are used by the bacteria to destroy various viral DNAs that might enter the cell, thereby restricting the potential growth of the virus.

Thus, restriction enzymes serve as defence mechanism. The bacteria protect its own DNA from nucleolytic attack by methylating the bases at susceptible sites, a chemical modification that blocks the action of the enzyme.

The restriction enzymes are thus the molecular scissors that are used to recognize and cut DNA at specific sequences. The sites recognized by them, are called **recognition sequences** or **recognition sites**. Different restriction enzymes found in different organisms recognize different nucleotide sequences and therefore cut DNA at different sites. Table encloses list of some restriction endonucleases and the site at which they cleave DNA.

Activity :

Find out the biological source of following restriction enzymes and discuss their recognition sequences :

Pst I, Sal I, Taq I, Xer III, Mbo II, Hpa I, BgII, Kpn I, Not I.

ii. Recognition sequences :

The sequences recognized by restriction enzymes are 4 to 8 nucleotides long and characterized by a particular type of internal symmetry. Consider the particular sequence recognized by the enzyme EcoRI .

Table 12.2 : Source and recognition sequences (indicated by arrow) of various restriction enzymes:

Restriction Enzyme	Source (Organism and strain)	Recognition sequence	Product	End products produced
Alu I	*Arthobacter luteus*	5' ---A- G-↓-C -T--- 3' 3' ---T- C-↑-G-A--- 5'	5'---A-G C-T---3' 3'---T-C G-A---5'	Blunt ends
Bam HI	*Bacillus amyloliquefaciens H*	5'---G-↓-G-A-T-C-C---3' 3'---C-C-T-A-G-↑-G---5'	5'---G G-A-T-C-C---3' 3'---C-C-T-A-G G---5'	Sticky ends
EcoRI	*Escherichia coli* Ry13	5'---G-↓-A-A-T-T-C---3' 3'---C-T-T-A-A-↑-G--- 5'	5'---G A-A-T-T-C---3' 3'---C-T-T-A-A G---5'	Sticky ends
Hind II	*H. influenzae* Rd	5'--G-T-C-↓-G-A-C--3' 3'--C-A-G-↑-C-T-G--5'	5'--G-T-C G-A-C--3' 3'--C-A-G C-T-G--5'	Blunt ends

3'------- C T T A A G-----5'

5'------- G A A T T C-----3'

When one reads the sequence in opposite direction (3' to 5' or 5' to 3') it is identical/ same.

DNA showing palindromic sequence

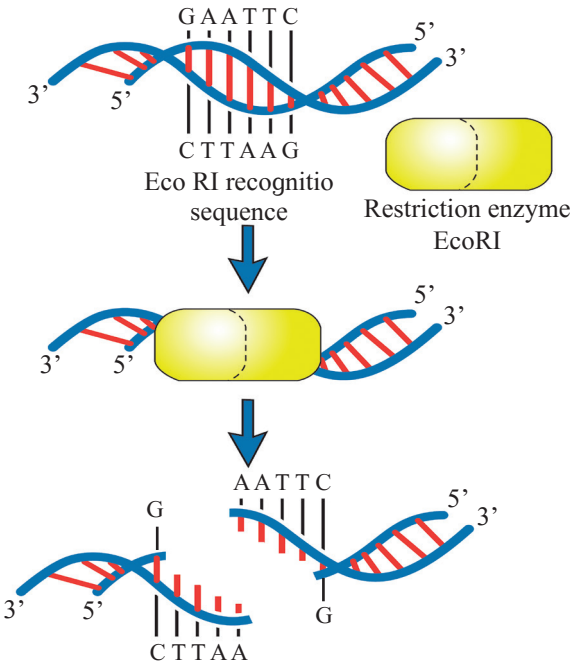

Fig. 12.3 : Mode of action of EcoRI to cleave DNA

A sequence with this type of symmetry is called a **palindrome**. When the enzyme EcoRI attacks this palindrome, it breaks each strand at the same site in the sequence, which is indicated by the arrow between the A and G residues.

3' ---- C T T ↓ A A G -------5'

5' ---- G A A ↑ T T C -------3'

Restriction enzymes either cut straight across the DNA in the region of palindrome to give blunt ends or cuts producing short, single stranded projections at each end of DNA to produce, cohesive or sticky ends or staggered ends.

B. Cloning vectors (vehicle DNA) - Vectors are DNA molecules that carry a foreign DNA segment and replicate inside the host cell. Vectors may be plasmids, bacteriophages (M13, lambda virus), cosmids, phagemids, BAC (bacterial artificial chromosome), YAC (yeast artificial chromosome), transposons, baculoviruses and mammalian artificial chromosomes (MACs). Most commonly used vectors are plasmid vectors (pBR 322, pUC, Ti plasmid) and bacteriophages (lamda phage, M13 phage). Plasmids and bacteriophages are most commonly used as vectors.

Do you know ?

Smith, Nathan and Arber achieved the discovery of restriction enzymes. For this spectacular achievement, they were awarded Nobel Prize for physiology and medicine in 1978. Since then restriction enzymes have been used for genetic manipulation by dissecting, analyzing and re-configuration of the genetic information at the molecular level.

Do you know ?

There are three types of restriction enzyme viz,

Type I - Which function simultaneously as endonuclease and methylase e.g. EcoK.

Type II - Which have separate activities for cleaving and methylation; they are more stable and are used in rDNA technology e.g. EcoRI, BgII; these enzymes cut DNA at specific sites within the palindrome.

Type III - Which cut DNA at specific non-palindromic sequences e.g. HpaI, MboII.

Thousands of type II REs. are recognized.

A good vector should have the ability of independent replication so that as the vector replicates (through *ori* gene), large number of copies of the DNA insert will be formed. Moreover the vector should be able to easily get introduced into host cells.

A vector should have marker genes for antibiotic resistance; must contain unique cleavage site in one of the marker genes for restriction enzyme; it should have at least suitable control elements like promoter, operator, ribosomal binding sites, etc. The plasmids obtained naturally do not posses all the characteristics. Hence, they are constructed by inserting gene for antibiotic resistance. e.g. pBR 322, pBR 320, pACYC 177 are the constructed plasmids. pBR 322 is mostly used in rDNA technology in plants.

i. **Plasmid :** The plasmids most commonly used in recombinant DNA technology are those that replicate in *E. coli.* Investigators have engineered these plasmids to optimize their use as vectors in DNA cloning.

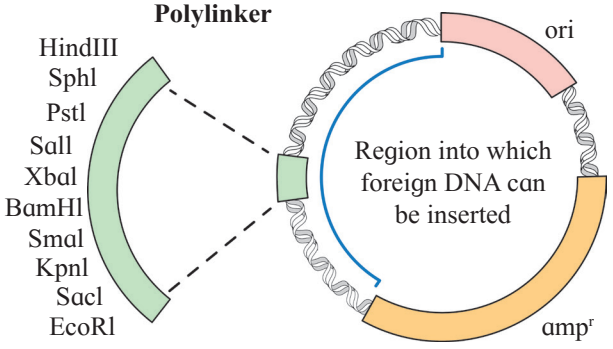

Fig. 12.4 : Plasmid cloning vector showing a replication origin (ori), a drug resistance gene (amp^r) and a region in which foreign DNA can be inserted.

ii. **Plasmid vectors for plants :** An important vector for carrying new DNA into many types of plants is a plasmid that is found in *Agrobacterium tumefaciens*. This bacterium lives in the soil and causes a plant disease called crown gall, which is characterized by the presence of over- growths, or tumors, in the plant. *A. tumefaciens* contains a plasmid called Ti (for tumor-inducing). The Ti plasmid contains a transposon, called T DNA, which inserts copies of itself into the chromosomes of infected plant cells. The transposon, with the new DNA, can still be inserted into the host cell's chromosomes. A plant cell containing this DNA, can then be grown in culture or induced to form a new, transgenic plant.

C. **Competent hosts** (cloning organisms) used are usually the bacteria like *Bacillus haemophilus*, *Helicobacter pyroli* and *E. coli*.

Mostly *E. coli* is used for the transformation with recombinant DNA

12.3 Methodology for rDNA technology :

The steps involved in gene cloning are as follows :

a. Isolation of DNA (gene) from the donor organism :

i. The desire gene to be cloned has to be obtained from the source organism (donor). Initially the cells of the donor organism are sheared with the blender and treated with suitable detergent. Genetic material from the donor is removed, isolated and purified by using several techniques. Isolated DNA can be spooled on to a glass rod.

ii. Isolated purified DNA is then cleaved by using restriction enzymes particularly Restriction Endonucleases (RE). These enzymes cleave DNA at specific sites, called **restriction sites** and break the DNA into fragments. There are several types of restriction endonucleases. Cleaved DNA fragments have cohesive, **sticky**, staggered ends or **blunt** ends.

From cleaved DNA fragments, a fragment containing desired gene is isolated and selected for cloning. This is now called **foreign DNA** or **passenger DNA**. A desired gene can also be obtained directly from genomic library or cDNA library.

Do you know ?

Gene library is a collection of different DNA sequences from an organism where each sequence has been cloned into a vector for ease of purification, storage and analysis. There are two types of gene libraries on the basis of the source of DNA used.

- Genomic library : It is a collection of clones that represent the complete genome of an organism. The genomic library of prokaryotes can be constructed by using plasmid vector. It is because prokaryotic genome does not contain repeatitive DNA.
- cDNA library : It represents the library of eukaryotic organisms only. DNA is produced from isolated mRNA by reverse transcription. The DNA so made is called complementary DNA (cDNA). The library is called cDNA library. Eukaryotic DNA genome contains introns, regulatary genes and repeatitive DNA. Hence, the establishment of genomic library in eukaryotes is not meaningful.

b. Insertion of desired foreign gene into a cloning vector (vehicle DNA) :

The foreign DNA or passanger DNA is now inserted into a cloning vector or vehicle DNA. The most commonly used cloning vectors are plasmids of bacteria and the bacteriophage viruses like lamda phage and M13. The most commonly used plasmid is pBR 322.

Plasmids are isolated from the vector organims i.e. bacterium. By using same RE (which is used in the isolation of the desired gene from the donor), plasmid i.e. vector DNA is cleaved.

Now by using enzyme DNA ligase, foreign DNA is inserted/ integrated into the vector DNA. The combination of vector DNA and foreign DNA is now called **Recombinant DNA** or **Chimeric DNA** and the technology is referred to as rDNA technology.

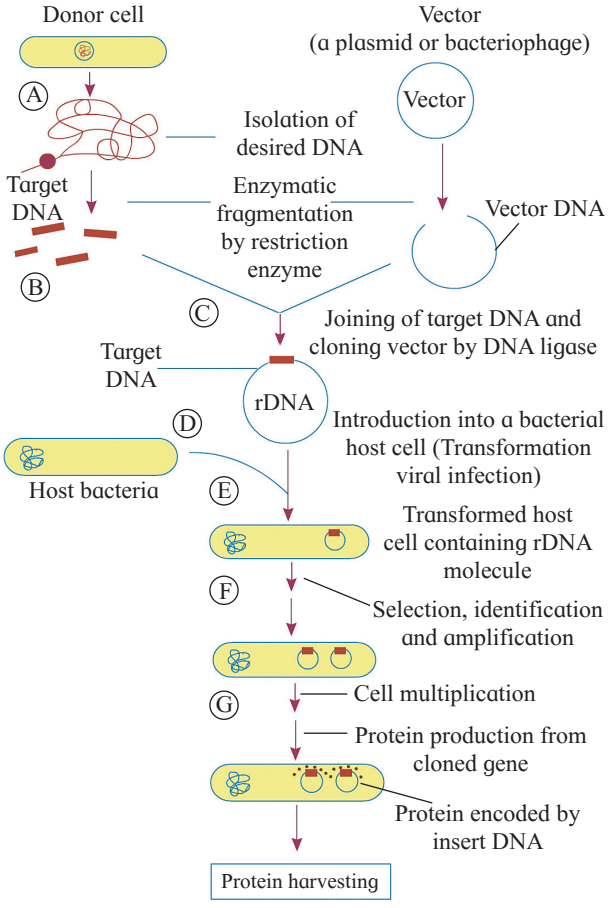

Fig. 12.5 : Outline of the process of recombinant DNA technology

c. Transfer of rDNA into suitable competent host or cloning organism :

Finally the recombinant DNA is now introduced i.e. transferred for expression into a competent host cell of the suitable cloning organism which is usually a bacterium. Host cell takes up naked rDNA by process of 'transformation' and incorporates into its own chromosomal DNA which finally expresses the trait controlled by passenger DNA. The transfer of rDNA into a bacterial cell is assisted by divalent Ca^{++}. The cloning organisms used in plant biotechnology are *E.coli* and *Agrobacterium tumifaciens*. The host/ competent cell which has taken up rDNA is now called **transformed cell**.

Foreign DNA can also be transferred directly into the naked cell or protoplast of the competent host cell, without using vector. This

is done by using techniques like electroporation, microinjection, lipofection, shot gun, ultrasonification, biolistic method, etc. But in plant biotechnology the transformation is through Ti plasmids of *A. tumifaciens*.

d. Selection of the transformed host cell :

The transformation process generates a mixed population of transformed (recombinant) and non-transformed (non-recombinant) host cells. For isolation of recombinant cell from non-recombinant cell, marker genes of plasmid vector is employed. For example, pBR322 plasmid vector contains different marker genes (Ampicillin resistant gene and Tetracycline resistant gene). When Pst1 RE is used, it knocks out Ampicillin resistant gene from the plasmid, so that the recombinant cell become sensitive to Ampicillin.

e. Multiplication of transformed host cell:

Once transformed, host cells are separated by the screening process. In this step the transformed host cells are introduced into fresh culture media.

At this stage the host cells divide and redivide along with the replication of the recombinant DNA carried by them.

f. Expression of the gene to obtain the desired product:

The next step involves the production of desired products like alcohol, enzymes, antibiotics, etc. Finally the desired product is separated and purified through downstream processing using suitable bioreactor.

Do you know ?

The Centre for Cellular and Molecular Biology (CCMB) located in Hyderabad, is a premier research organization in frontier areas of modern biology including DNA fingerprinting and Molecular approaches in animal breeding. The objectives of the Centre are to conduct high quality basic research and training in frontier areas of modern biology, and promote centralized national facilities for new and modern techniques in the inter-disciplinary areas of biology.

12.4 Applications of Biotechnology:

Biotechnology is an umbrella term covering a broad spectrum of scientific applications used in many sectors, such as health and agriculture, industry, environment and genomics.

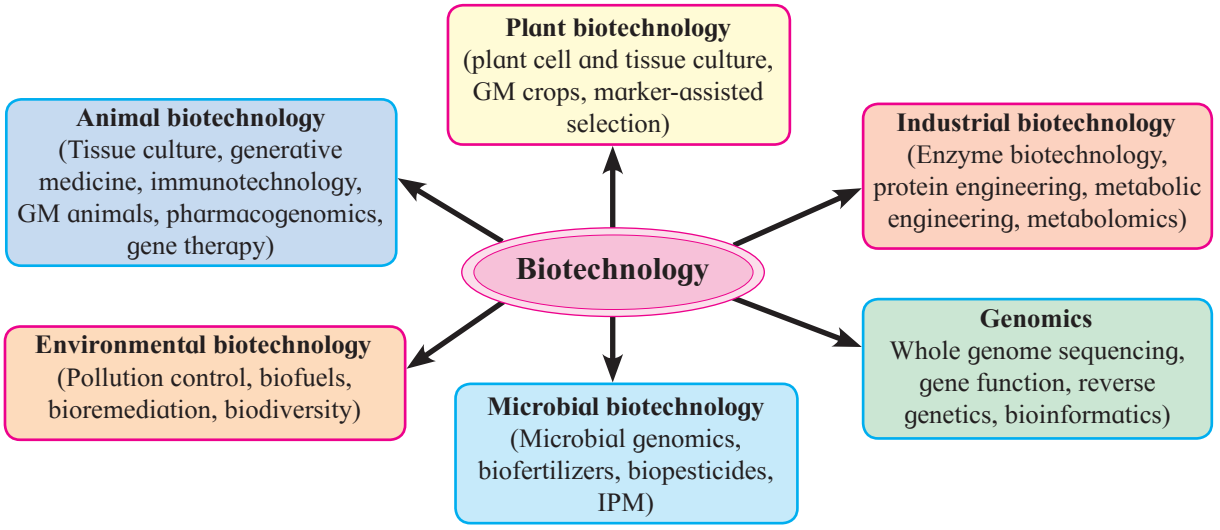

Fig. 12.6 : Applications of Biotechnology

Tabel 12.7 : Human proteins produced by rDNA technology to treat human diseases

Disorder/ Diseases/ Health condition	Recombinant protein(s)
1. Anaemia	Erythropoeitin
2. Asthma	Interleukin-1 receptor
3. Atherosclerosis	Platelet derived growth factor
4. Parturition	Relaxin
5. Blood clots	Tissue Plasminogen Activator (TPA) Urokinase
6. Cancer	Interferons, tumour necrosis factor interleukins, macrophage activating factor
7. Diabetes	Insulin
8. Emphysema	α_1- Antitrypsin
9. Haemophilia A	Factor VIII
10. Haemophilia B	Factor IX
11. Hepatitis B	Hepatitis B vaccine

a. Healthcare Biotechnology :

It refers to a medicinal or diagnostic product or a vaccine. This technology has a tremendous impact on meeting the needs of patients. Biotechnology offers patients a variety of new solutions such as: unique, targeted and personalized therapeutic and diagnostic solutions for organ transplant. Stem cell technology, genetic counselling, forensic medicine, gene probes, genetic fingerprinting and karyotyping are the outcomes of biotechnology.

Human insulin :

Insulin is a peptide hormone produced by β-cells of islets of Langerhans of pancreas. It was discovered by Sir Edward Sharpey Schafer (1916) while studying Islets of Langerhans.

Insulin is essential for the control of blood sugar levels. Diabetes mellitus is a disease in which some people cannot make insulin themselves. Hakura et al (1977), chemically synthesized DNA sequence of insulin for two chains A and B and separately inserted into two pBR322 plasmid vector. Insulin production by recombinant DNA technology is designed by Gilbert and Villokomaroff in 1978.

The genes are inserted by the side of β-galactosidase gene of the plasmid. The recombinant plasmids were then separately transformed into *E. coli* host. The host produced penicillinase + pre-pro insulin. Insulin is later separated by trypsin treatment.

Vaccine production:

A vaccine is a biological preparation that provides active acquired immunity against a certain disease. Usually a vaccine consists of a biological agent that represents the disease-causing microorganism. It is often made from a weakened or killed form of the microorganism, its toxins or one of its surface protein antigens.

Biotechnology has offered modern diagnostic test kits-rickettsial, bacterial and viral vaccines along with radiolabelled biological therapeutics for imaging and analysis.

Vaccines have eliminated small pox, polio and other deadly diseases for the last several decades. Biotechnology has made advancements in vaccination by making recombinant vaccines that have the potential to eradicate non-communicable diseases like cancer. Naked DNA vaccines, viral vector vaccines and plant-derived vaccines are found to be most effective against a number of bacterial and viral disorders.

Internet my friend

Collect the information pertaining to recombinant vaccines and their types - protein vaccines and DNA vaccines.

Oral vaccines: a novel approach :

The latest hot spot in the field of vaccine research is the development of vaccine which can be taken orally. Immunogenic protein of

certain pathogens is found to be active when administered orally. The gene corresponding to such proteins is isolated and a gene construct is produced. This is introduced and expressed in a plant genome, which results in production of such immunogenic proteins in the parts of the plant where it is expressed. These when fed into animals or mainly humans, the person becomes vaccinated against certain pathogen. Such vaccines are also known as **edible vaccines**.

An exciting invention is production of *'melt in the mouth'* vaccines that can be administered by placing them under your tongue that delivers it into the blood stream. The most important example is the production of flu vaccine by *Bacillus* which melts in the mouth. The tremendous benefit of such vaccines, is the comfort of administration, low cost and ease of storage.

b. Agriculture :

Application of Biotechnology in Agriculture involves scientific techniques such as Genetically Modified Organisms, Bt Cotton, Pest Resistant Plants. It helps in modifying plants, animals, and microorganisms and improve their agricultural productivity.

Tissue Culture is used in Micropropagation i.e. large-scale propagation of plants in very short durations. Tissue culture technique is also the best method for storing germplasm and maintaining a specific genetic type (Clone). This technique is used in those plants, which produce recalcitrant seeds or produce highly variable seeds.

Recalcitrant means the reduction in the seed moisture contents below certain levels and freezing drastically reduces the survival and thus present difficulty in storage. Here, subcellular damage of seeds occur accompanied by consequent loss of viability, when dried.

c. Gene therapy:

A gene is a stretch of DNA required to make a functional product such as part or all of a protein. During gene therapy, DNA that codes for specific genes is delivered to individual cells in the body.

Gene therapy is the treatment of disease by replacing, altering or supplementing a gene that is absent or abnormal and whose absence or abnormality is responsible for the disease.

Most, if not all, diseases have a genetic factor. The genetic factor can be wholly or partially responsible for the disease. For example, in disorders such as cystic fibrosis, haemophilia, and muscular dystrophy, changes in a gene directly result in the condition.

In other conditions, such as high cholesterol and high blood pressure, genetic and environmental factors interact to cause disease. There are more than 5000 different human genetic diseases known to be caused by single gene defects e.g. sickle cell anaemia, thalassemia, Tay-sach's disease, cystic fibrosis, Huntington's chorea, haemophilia, alkaptonuria, albinism, etc.

Gene therapy is being used in many ways. For example, to:

- Replace missing or defective genes;
- Deliver genes that speed the destruction of cancer cells;
- Supply genes that cause cancer cells to revert back to normal cells;
- Deliver bacterial or viral genes as a form of vaccination;
- Deliver DNA to antigen expression and generation of immune response;
- Supply of gene for impairing viral replication;
- Provide genes that promote or impede the growth of new tissue; and
- Deliver genes that stimulate the healing of damaged tissue.

Do you know ?

Delivery of genes into cells :

Genes can be delivered by three ways :

i. *Ex vivo* delivery where cells are removed from the patients and then gene is introduced using viral or non-viral vectors e.g. **Parkinson's disease**, a neurological disorder.

ii. *In vivo* delivery where therapeutic genes are directly delivered in the cells at the target sites of the diseased tissue in the patient- like intravenous infusion genes to treat cancer are injected directly into tumor.

iii. Use of virosomes (Liposome + inactivated HIV), bionic chips are the other methods of gene delivery.

Forms of gene therapy :

a. Germ line gene therapy : In this method healthy genes can be introduced into germ cells like sperms, eggs, early embryos. It allows transmission of the modified genetic information to the next generation. Though it is highly effective in counteracting the genetic disorders, it is not encouraged for application in human beings due to a variety of technical and ethical reasons.

b. Somatic cell gene therapy : In this type the gene is introduced only in somatic cells like bone marrow cells, hepatic cells, fibroblasts endothelium and pulmonary epithelial cells, central nervous system, endocrine cells and smooth muscle cells of blood vessel walls. Modification of somatic cells only affects the person being treated and the modified chromosomes cannot be passed on the future generations. Somatic cell gene therapy is the only feasible option and the clinical trials have already employed for the treatment of acquired disorders such as cancer and rheumatoid arthritis and blood disorders including SCID, Gaucher's disease, familial hypercholesterolemia, haemophilia, phenylketonuria, cystic fibrosis, sickle cell anaemia, Duchenne muscular dystrophy, emphysema, thalassemia etc.

d. Genetically Modified Organisms (GMOs):

These are living organisms whose genetic material has been artificially manipulated in a laboratory through genetic engineering. This creates combinations of plant, animal, bacteria, and virus genes that do not occur in nature or through traditional crossbreeding methods.

Most GMOs have been engineered to withstand the direct application of herbicide and/or to produce an insecticide. However, new technologies are now being used to artificially develop other traits in plants, such as a resistance to browning in apples, and to create new organisms using synthetic biology. Despite biotech industry promises, there is no evidence that any of the GMOs currently on the market, offer increased yield, drought tolerance, enhanced nutrition, or any other consumer benefit.

I. Transgenic Plants :

The human race is very dependent on agriculture and as world populations continue to expand, there must be continuous reassessment of agricultural practices to optimize their efficiency. Since early times human beings have sought to improve the quality and productivity of agriculturally important plants. This was done by selection and traditional breeding procedures that were painstakingly slow and difficult. Traditional breeding programmes involve sexual crosses, which resulted in the high quality of present day food plants such as wheat, rice, corn, potato, etc. More recently, biotechnological approaches have been applied to these plants to create genetic variations that are beneficial for mankind.

First transgenic plant produced was tobacco. More then 60 transgenic dicot plants and several monocot plant like maize, oat,

rice, wheat are known. Tomato, soybean, potato, sugar beet, grapes, brinjal, cotton are other transgenic plants. Transgenic plants are being looked up as bioreactors for molecular farming i.e. for production of novel drugs like interferons, edible vaccines, antibodies, amino acids, immunotherapeutic drugs, etc.

Advantages of GM food-plants :

The ways in which one thinks that genetically modified plants can help, are listed as follows:

a. Insect pest resistance:

It can help farmers to reduce their use of chemical pesticides, which in turn can reduce the cost of producing food. However, an alternative has been available for more than 30 years which is a biological insecticide from the bacterium, *Bacillus thuringiensis* (Bt). However, the use of *B. thuringiensis* sprays is limited because of low stability of the protein in the field.

Bt cotton is one of the best transgenic plants known for its insect resistance property.

Insect resistant plants contain either a gene from *B. thuringiensis* or the cowpea trypsin inhibitor gene. The gene called cry gene present in *B. thuringiensis* produces a protein that forms crystalline inclusions in bacterial spores. When ingested by a susceptible insect, a combination of high pH and the enzyme proteinase of the insect's midgut, processes them hydrolytically to release the core toxic fragments.

The effect of these fragments is seen within minutes of ingestion, beginning with midgut paralysis and ending with disruption of midgut cells of insect. *Bt* toxin activity has been against many species of insects within the orders of Lepidoptera, Diptera, and Coleoptera.

Golden rice - a transgenic food crop used to reduce vitamin A deficiency disease.

Similarly, the gene of α-amylase inhibitor (αAl-Pv) has been isolated from adzuki bean (*Phaseolus vulgaris*) and transferred to tobacco and this gene works against pests like *Zabrotes subfasciatus* and *Callosobruchus chinensis*.

b. Improved nutritional qualities (biofortification):

Transgenic plants have also been produced to provide functional food and neutraceuticals. For millions of people in developing countries, rice is the main item in their diet. Because rice does not contain many essential nutrients, malnutrition is very common in these countries.

Especially terrible is the blindness that results from a lack of vitamin A. This vitamin is abundant in milk and in vegetables such as carrots, which most of the poor people of the world cannot afford. To solve this problem, Swiss researchers created transgenic rice (golden rice) and transgenic mustard (golden mustard) varieties that are high in vitamin A. The golden colour is due to vitamin A. They hoped that this rice, if grown and eaten in developing countries, would reduce the diseases associated with vitamin A deficiency (VAD).

Table 12.8 : Some transgenic plants produced for functional food and neutraceuticals

Substance	Potential benefit	Crop	Transgene
Provitamin A	Anti-oxidant	Rice	Phytoene synthase, lycopene cyclase
Vitamin E	Anti-oxidant	Canola	γ- tocopherol methyl trasnferase
Flavonoids	Anti-oxidants	Tomato	Chalone isomerase
Fructants	Low calories	Sugarbeet	1-sucroese: sucrase fructosyl transferase
Iron	Iron fortification	Rice	Ferritin, metallothioein, phytase

Improvement in oil content and oil quality of oil crops like soybean, oil palm, rapeseed and sunflower, have been achieved by transfer of '*Arabidopsis* genes'.

Iron deficiency is also a serious nutritional problem, affecting an estimated 30% of the world population. For production of transgenic crops that will produce food rich in iron, an iron storage protein (ferritin) is targeted. Ferrritin is found in many animals, plants and bacteria. The genes for ferritin protein isolated from soybean and *Phaseolus* have been transferred to rice.

Do you know ?

- Genetically engineered **herbicide tolerant** plants are developed as in maize, wheat and many other monocot plants.
- Genetically engineered **disease resistant plants** (against bacterial and viral pathogens) are also developed in crop plants like tomato, potato and tobacco.
- Plants deficient in amino acids like methionine, lysine and tryptophan are genetically engineered by introducing genes from other sources so as to make the seeds protein rich. e.g. Leguminous plants (pulses), maize, etc.
- Similarly, genetically engineered plants tolerant to abiotic stresses such as high temperature, water, cold, etc. are also developed.

c. Modification in Post-harvest characteristics:

Diseases and pests, bruising on soft fruits and vegetables, heat and cold storage, over-ripeness, loss of flavours and odours, etc. lead to great deal of losses during storage and transport of crops.

Most of these physiological changes are due to endogenous enzyme activity. Genetic engineering has made it possible to slow down these activities. In the tomato the enzyme polygalacturonase breaks down the cell wall constituent- pectin, leading to softening of fruit during ripening. Thus, the fruits are easily bruised and damaged on shipment. By inhibiting the polygalacturonase by antisense genes, the tomato (genetically modified tomatoes are called Flavr savr tomatoes) can remain on the vine until mature and be transported in a firm solid state.

d. Plants as factories :

To produce novel biochemicals and vaccines **(Biopharmaceuticals)**, plants are potential factories or bioreactors for high value biochemicals like starch, sugar, lipids, proteins, and products like fine chemicals, perfumes and adhesive compounds as well as industrial lubricants, biodegradable plastic and even 'renewable' energy crops to replace fossil fuels.

Biopharmaceuticals are proteins, hormones, antibodies, vaccines or enzymes isolated from transgenic plants. Some of the proteins that are being produced by transgenic crop plants:

- Human growth hormone with the gene inserted into the chloroplast DNA of tobacco plants.
- Humanized antibodies against such infectious agents as HIV, Respiratory syncytial virus (RSV), Herpes simplex virus (HSV), the cause of "cold sores"
- Protein antigens to be used in vaccines for e.g. Patient-specific antilymphoma (a cancer) vaccines. B-cell lymphomas are clones of malignant B cells expressing on their surface a unique antibody molecule.

Currently many novel products have been commercially exploited for their products such as:

a. A 'superglue' produced by tobacco plants with genes encoding for powerful adhesive proteins, enables marine mussels to stick to rocks. It will be especially valuable as a biochemical glue for body repairs during surgery.
b. Transgenic plants, containing oil-encoding gene from marine algae, produce oil that has nutritional value similar to cod- liver oil.
c. Plant that will produce the antimalarial drug, Artemisinin.
d. Genetically engineered opium poppy to produce more powerful painkillers.

e. Transgenic plants producing edible vaccines:

Genetically altered plants can provide protection to infectious diseases. Plant products acting as vaccines would be inexpensive to produce and thus, can easily be made available in developing countries. Potatoes, tomatoes, bananas, soybeans, alfalfa and cereals are the most common foods proposed for edible vaccine delivery.

II. Transgenic animals:

Many transgenic animals such as mice, rats, rabits, pig, sheep, cows, fowls, fish have been produced through rDNA technology. The term transgenic animal refers to an animal in which there has been a deliberate modification of the genome - the material responsible for inherited characteristics - in contrast to spontaneous mutation. Foreign DNA is introduced into the animal, using recombinant DNA technology, and then must be transmitted through the germ line so that every cell, including germ cells, of the animal contain the same modified genetic material.

A representative, but non-inclusive, list of purposes for which transgenic animals have been used, indicates the wide-ranging application of this biotechnology:

- in medical research, transgenic animals are used to identify the functions of specific factors in complex homeostatic systems through over- or under-expression of a modified gene (the inserted transgene);
- in toxicology: as responsive test animals (detection of toxicants);
- in mammalian developmental genetics;
- in molecular biology, the analysis of the regulation of gene expression makes use of the evaluation of a specific genetic change at the level of the whole animal;
- in the pharmaceutical industry, targeted production of pharmaceutical proteins, drug production and product efficacy testing;

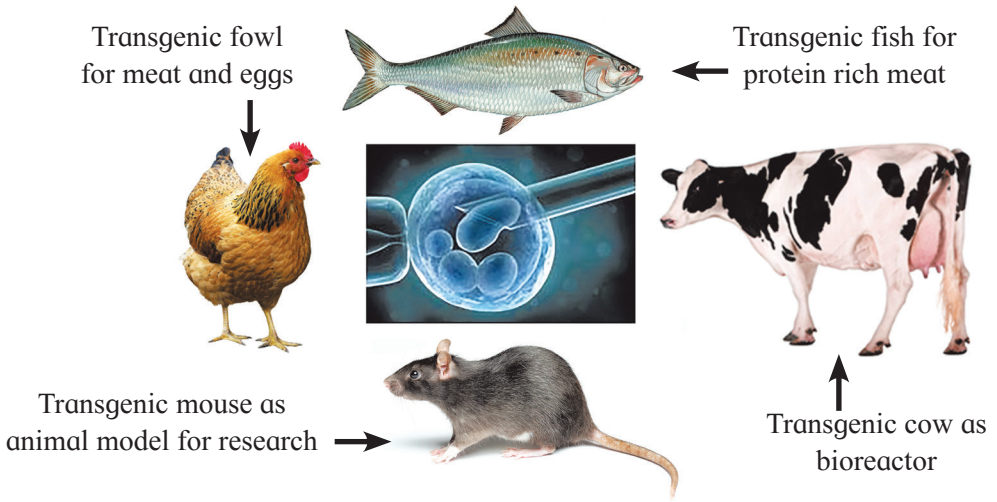

Fig. 12.9 : Transgenic animals

- in biotechnology: as producers of specific proteins;
- genetically engineered hormones to increase milk yield, meat production; genetic engineering of livestock and in aquaculture affecting modification of animal physiology and/or anatomy; cloning procedures to reproduce specific blood lines; and
- developing animals specially created for use in xenografting.

a. Transgenic mice and cancer research :

Through laboratory investigations with transgenic mice that have been modified using a particular oncogene (cancer causing gene) and thus developed a certain type of cancer, questions concerning the relationship between oncogenes and cancer development could be answered. Theoretically, such animals can also be used for research into cancer treatment and prevention of malignancy.

In the laboratory of Philip Leder in Harvard (USA) the transgenic mouse model for the investigation of the breast cancer was developed. The oncogenes *myc* and *ras* were analysed to find out if they lead to breast cancer in mice transformed with these genes.

b. Transgenic farm animals :

With the advent of technology, the intention of researchers is diverted to produce transgenic farm animals from which the mankind can derive greater benefits. Many of the farm animals are improved for their meat production ability while some of them are improved for milk yields and quality, and disease-free status. At the beginning of the century, a dairy cow provided 2,000 to 3,000 liters of milk a year.

Today, Holstein cow provides 6,000 liters on average and up to 8,000 – 10,000 for the best ones. A century ago, a hen laid about 70 eggs a year whereas today the best races lay up to 250 eggs per year. This could be possible because of the advent of biotechnology.

The main objectives for improved animal breeding programmes coupled with this new technology of gene transfer are given below :

- Efficiency of meat production
- Improved quality of meat
- Milk quality and quantity
- Egg production
- Wool quality and quantity
- Disease resistance in animals
- Production of low-cost pharmaceuticals and biologicals

c. Transgenic cattle for food production :

Of the few research reports describing the use of transgenic technologies in cattle only one is directed towards a food production application. Researchers introduced additional copies of bovine beta or kappa casein into dairy cattle and evaluated the effect on milk production and composition. Transgenic offspring had an 8 to 20% increase in beta casein and a two-fold increase in kappa casein.

d. Transgenic cattle for human therapeutic production :

A second application for genetically modified cattle is the production of human therapeutic proteins. Human proteins that have been expressed in milk include human lactoferrin, human alpha lactalbumin, human serum albumin and human bile salt stimulated lipase. The mammary gland in dairy cows is an excellent protein production factory. On the other hand, one transgenic cow would be more than sufficient for production of annual world supply of factor IX (plasma thromboplastin component) that is used in the treatment of haemophilia.

In 1990 Tracy, the transgenic cow was born in Scotland, and could produce a human protein in her milk for human therapeutics.

Antibodies are currently used for many different human clinical applications; including treatment of infectious disease, cancer,

transplanted organ rejection, autoimmune diseases and for use as antitoxins. To make a human antibody product, the genetically modified cows are immunized with a vaccine containing the disease agent.

e. Transgenic Sheep :

Gene transfer technology is applied to sheep to produce transgenic sheep which are able to achieve better growth and meat production as well as to serve as bioreactors. Human growth hormone gene is introduced in sheep for promoting the growth and meat production.

Bacterial genes, *cys E* and *cys M*, are concerned with biosynthesis of cysteine amino acids involved in formation of keratin protein found in wool. Both these genes are identified, cloned and introduced in sheep to increase wool production and to improve the quality of wool.

f. Transgenic pigs :

The objective of gene transfer in pigs is to increase growth and meat production and to act as bioreactors.

Pigs are regarded as the most suitable animals to be bred for heart transplant because a pig's heart is about the same size as a human heart, and pig heart valves have been used in human heart surgery for over a decade. The pig clone is the first step towards providing animal organs and tissues for human transplants (xenotransplantation).

g. Transgenic fish :

The commercially important fish like Atlantic salmon, catfish, goldfish, *Tilapia*, zebra-fish, common carp, rainbow trout, etc. are transfected with growth hormone, chicken crystalline protein and *E.coli* hygromycin resistance gene. Transgenic fish showed increased cold tolerance and improved growth and it is the quantity and quality of fish proteins as well as its preservation, are the factors affecting the economic value of fish.

h. Transgenic chicken :

Also carry and express foreign genes. They could be used to improve the genetic make-up of existing strains with respect to built-in (*in vivo*) resistance to viral and coccidial diseases, better feed efficiency, lower fat and cholesterol levels and high protein containing eggs, and better meat quality.

12.5 Bioethics, bio-piracy and bio-patent :

Bioethics:

Ethics usually deals with the matters related to socially acceptable moral duty, conduct and judgement. In other words, it helps to regulate the behaviour of cummunity by some set of standards. However the concepts differ according to culture and traditions. Moreover concepts change with the time due to shifting of perception of values which are affected by progress in science and technology. Bioethics helps to study moral vision, decision and policies of human behaviour in relation to biological phenomena or events. Ethics deals with 'Life' e.g. *in vitro* fertilization, sperm bank, gene theropy, cloning, gene manipulations, euthanasia, death, maintaining those who are in comatose state, prenatal genetic selection, etc.

The era of biotechnology has brought wide spectrum on new topics like cloning, transgenic, gene therapy, eugenics, rDNA technology, etc. The use of all these has drawn a wide range of reactions in the society. The reactions are based on individual's own perception and moral. Ethical aspects pertaining to the use of biotechnology seems to be more controversial and frightening. These concerns are broadly summarized below :

Use of animals causes great sufferings to them; voilation of integrity of species caused due to transgenosis; transfer of human genes into animals and vice versa; indiscriminate use of biotechnology pose risk to the environment, health and biodiversity.

The introduction of Genetically Modified Organisms (GMOs) has led to a wider debate on bioethical concerns affecting social, economic and environmental spheres. These include the effects on non-target organisms, insect resistance crops, gene flow and the loss of diversity as well as the issue on interfering with nature in which the modification process itself is disrupting the natural process of biological entities. Ethics in biotechnology also includes the general subject of what should and should not be done in using recombinant DNA techniques

12.6 Effects of Biotechnology on the Environment :

a. Herbicide Use and Resistance :

Effects on the environment are a particular concern with regard to GMO crops and food production. One area of development involves adding the ability to produce pesticides and resistance to specific herbicides. These traits are helpful in food production, allowing farmers to use fewer chemicals, and to grow crops in less than ideal conditions. However, herbicide use could be increased, which will have a larger negative effect on the surrounding environment. Also unintended hybrid strains of weeds and other plants can develop resistance to these herbicides through cross-pollination, thus negating the potential benefit of the herbicide. One such herbicide that has already been added is RoundUp. Crops of RoundUp-ready soybeans have already been implemented into agricultural practices, possibly conferring RoundUp resistance to neighboring plants.

b. Effects on Untargeted Species :

Bt corn, which produces its own pesticide, is also in use today. It has adverse effects on Monarch butterfly populations, which are not the original target of the pesticide. It can also have unintentional effects on neutral or even beneficial species.

12.7 Effects of Biotechnology on Human Health :

a. Allergies :

GMO crops could potentially have negative effects on human health as well. Consumers have developed unexpected allergic reactions. e.g. Researchers used a gene from the Brazil nut to increase the production of Methionine in soybeans. The insertion of this gene inadvertently caused allergic reactions to the soybean in those with known nut allergies ("Biotech Soybeans").

b. Long-Term Effects :

Because GMO technology has been available for such a short amount of time, there is relatively little research which has been conducted on the long-term effects on health which we cannot anticipate at this point.

c. New Proteins :

Proteins that have never been ingested before by humans are now part of the foods that people consume every day. Their potential effects on the human body are as of yet unknown.

d. Food Additives :

GMOs also present us with possibilities of introducing additional nutrients into foods, as well as antibiotics and vaccines. This availability of technology can provide nutrition and disease resistance to those countries that don't have the means to provide these, otherwise.

However, there is possibility of the creation of antibiotic and vaccine-resistant strains of diseases.

This shows that the vast advances in life sciences and our multicultural and pluralistic modern societies create numerous bioethical problems requiring some stringent regulation. In terms of GMOs, the Indian Government has set up the **Genetic Engineering Approval Committee (GEAC)**. This organization makes decisions regarding the validity of research involving GMOs and addresses the safety of GMOs introduced for public use.

12.8 Biopatent and Biopiracy :

a. Biopatent :

Patent is a special right granted to the inventor by the goverment. Patent is a personal property of inventor. It can be sold like any other property. A patent consists of three parts - **grant** (aggrement with the inventor), **specification** (subject matter of invention) and **claims** (scope of invention to be protected).

Biopatent is a biological patent. Biopatents are awarded for strains of microorganisms, cell lines, genetically modified strains, DNA sequences, biotechnological processes, product processes, product and product applications.

Biopatents are awarded to recognize real innovative contributions made by the inventor to the cause of human welfare. The awards are given to inculcate encouragement and values in developing scientific culture and in emphasizing the role of biology in shaping human society.

Indian patent allows 'process patent' and not the 'product patent'. Biopatent allows the patent holder to exclude others from making, using, selling or importing protected invention for a limited period of time. Duration of biopatents is five years from the date of the grant or seven years from the date of filing the patent application, which ever is less.

First biopatent was patented pertaining to genetically engineered bacterium '*Pseudomonas*' used for clearing oils spills. Patent under the title 'control of plant gene expression' was issued jointly to Delta and Pineland company and U. S. department of agriculture. Patent is based on a gene that produces a protein toxic to plant thus, do not allow seeds to germinate. However, this patent was not granted by Indian goverment. Such a patent is considered morally unacceptable and fundametally unequitable. This is because financially powerful corporations would aquire monopoly over biotechnological process. This in turn would pose a threat to global food security.

b. Biopiracy :

Pirates in general terms are those who steal and kill others to enrich themselves. Biopirates are those who do not kill but steal the patent (misuse the patent). Biopiracy is defined as 'theft of various natural products and then selling them by getting patent without giving any benefits or compensation back to the host country'. In short, it is unauthorized misappropriation of any biological resource and indigenous knowledge.

The developed, industrialized and financially rich nations are poor in biodiversity and traditional knowledge whereas developing and underdeveloped nations have ample of biodiversity and traditionally, they know better the use of their bio-resources. Traditional knowledge naturally includes a deep understanding of ecological processes and the ability to sustainably extract useful products from the local habitat. Traditional knowledge is handed over through the generations. This helps them to develop modern, commercial applications that save the makers time, money and effort.

Components of **Traditional Knowledge** that are especially relevant to our global survival include knowledge of:

- Food, crop varieties and agricultural/ farming practice
- Sustainable management of natural resources and conservation of biological diversity
- Biologically important medicines

The conservation of species, habitat, and biodiversity are essential to the continued survival of tribal people. By conserving the customs and habitat of tribal people, we concurrently reduce emissions from deforestation and ecosystem degradation. Furthermore, the opportunity for cultural survival is a basic human right. The traditional knowledge is facing a problem of biopiracy.

The act of Piracy is unauthorized publication or reproduction of another person's work or material. When someone indulges in piracy, the accused is using someone's work illegally or without taking any permission. The innovations and discovery of the pharmaceutical and agricultural researches are not new as to qualify as invention as they are based on centuries of knowledge of the traditional societies.

Examples of Biopiracy :

• Patenting of Neem (*Azadirachta indica*) :

The people of India in a variety of ways have used neem, since time immemorial. Indians have shared the knowledge of the properties of the neem with the entire world. Pirating this knowledge, the USDA and an American MNC W.R. Grace in the early 90s sought a patent from the European Patent Office (EPO) on the "method for controlling on plants by the aid of hydrophobic extracted neem oil." The patenting of the fungicidal properties of Neem, was an example of biopiracy.

• Patenting of Basmati :

Basmati is a long-grained, aromatic variety of rice indigenous to the Indian subcontinent.

In 1997 the US Patent and Trademark Office (USPTO) granted a patent to a Texas based American company Rice Tec Inc for "Basmati rice line and grains" having trade name **Texmati**. The patent application was based on 20 very broad claims on having "invented" the said rice. Due to peoples movement against Rice Tec in March 2001, the USPTO has rejected all the claims.

• Haldi (Turmeric) Biopiracy :

Two American researchers of Indian origin of the University of Mississippi Medical Center, put a claim to the US Patent and Trademark Office, maintaining that they had discovered *haldi*'s healing properties. Surprisingly, they were granted a patent in March 1995 for something you had known for years and our ayurvedas for centuries.

It meant they had exclusive rights over any such *haldi* drug and were in a position to make millions of dollars. The Council of Scientific and Industrial Research (CSIR) applied to the US Patent Office for a re-examination and they realized the mistake and cancelled the patent. This was after Indian scientists shouted from rooftops about how we are losing our traditional knowledge to marauding foreign companies who have started poaching on our ancient healing techniques.

It is the need of hour to launch genetic literacy movement in Indian school and colleges for better understanding of opportunities and risks related to biotechnology and also to promote the safe and meaningful use of technologies of modern life sciences.

Activity :

Collect information on the use of Biotechnology in pollution control.

Exercise

Q. 1 Choose the correct option

1. The bacterium which causes a plant disease called crown gall is .....
   b. *Helicobacter pylori*
   c. *Agrobacterium tumifaciens*
   d. *Thermophilus aquaticus*
   e. *Bacillus thuringienesis*

2. The enzyme nuclease hydrolyses ..... of polynucleotide chain of DNA.
   a. hydrogen bonds
   b. phosphodiester bonds
   c. glycosidic bonds
   d. peptide bonds

3. In vitro amplification of DNA or RNA segment is known as .....
   a. chromatography
   b. southern blotting
   c. polymerase chain reaction
   d. gel electrophoresis

4. Which of the following is the correct recognition sequence of restriction enzyme *Hin d* III.
   a. $^{5'}$---A-A-G-C-T-T---$^{3'}$
      $^{3'}$---T-T-C-G-A-A---$^{5'}$
   b. $^{5'}$---G-A-A-T-T-C---$^{3'}$
      $^{3'}$---C-T-T-A-A-G---$^{5'}$
   c. $^{5'}$---C-G-A-T-T-C---$^{3'}$
      $^{3'}$---G-C-T-A-A-G---$^{5'}$
   d. $^{5'}$---G-G-C-C---$^{3'}$
      $^{3'}$---C-C-G-G---$^{5'}$

5. Recombinant protein ..... is used to dissolve blood clots present in the body.
   a. insulin
   b. tissue plasminogen activator
   c. relaxin
   d. erythropoietin

6. Recognition sequence of restriction enzymes are generally ..... nucleotide long.
   a. 2 to 4 b. 4 to 8
   c. 8 to 10 d. 14 to 18

Q. 2 Very short answer type questions.

1. Name the vector which is used in protuction of human insulin through recombinent DNA technology.
2. Which cells from Langerhans of pancreas do produce a peptide hormone insulin?
3. Give the role of Ca^{++} ions in the transfer of recombinent vector into bacterial host cell.
4. Expand the following acronyms which are used in the field of protechnology.
   i. YAC ii. RE iii. dNTP
   iv. PCR v. GMO vi. MAC
5. Fill in the blanks and complete the chart.

GMO	Purpose
i. Bt cotton	.....
ii.	Delay the softening of tomato during ripening.
ii. Golden rice	.....
iv. Holstein cow	.....

Q. 3 Short answer type questions.

1. Explain the properties of a good or ideal cloning vector for rDNA technology.
2. A PCR machine can rise temperature upto 100 ^{0}C but after that it is not able to lower the temperature below70 ^{0}C automatically. Which step of PCR will be hampered first in this faulty machine? Explain why?
3. In the process of rDNA technology, if two separate restriction enzymes are used to

cut vector and donor DNA then which problem will arise in the formation of rDNA or chimeric DNA? Explain.

4. Match and write the pairs.

Recombinant protein	It's use in or for
i. platelet derived growth factor	a. Anemia
ii. α-antitrypsin	b. cystic fobrosis
iii. Relaxin	c. Haemophilia A
iv. Erythropoietin	d. Diabetes
v. Factor VIII	e. Emphysema
vi. DNA ase	f. Parturition
	g. Atherosclerosis

Q. 4 Long answer type questions.

1. Define and explain terms.
   i. Biopiracy ii. Biopatent
   iii. Bioethus
2. Explain the steps in process of rDNA technology with suitable diagrams.
3. Explain the gene therapy. Give two types of it.
4. How are the transgenic mice used in cancer research?
5. Give the steps in PCR or polymerase chain reaction with suitable diagrams.
6. What is a vaccine? Give advantages of oral vaccines or edible vaccines.
7. Enlist different types of restriction enzymes commonly used in rDNA technology? Write on their role.
8. Enlist and write in breif about the different biological tools required in rDNA technology.

Project :

Visit the tissue culture laboratory in your area. Prepare powerpoint presentation on tissue culture methodology and its applications.