Biotechnology and its application
(As per Maharashtra syllabus)

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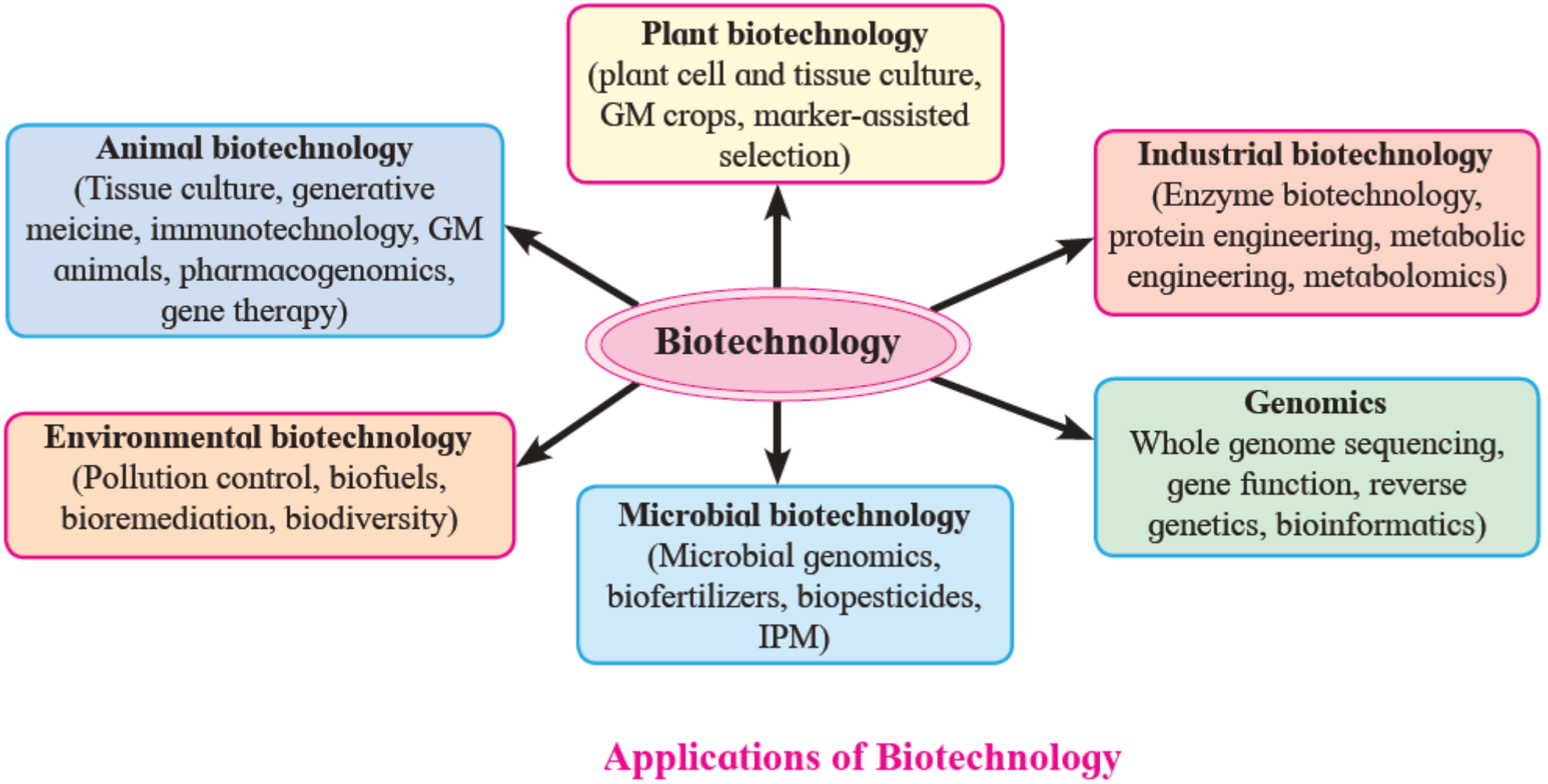
Applications of Biotechnology



Healthcare Biotechnology

It refers to a medicinal or diagnosticproduct or a vaccine

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.
- It is often made from a weakened or killed form of the microorganism, its toxins or one of its surface protein antigens.
- Naked DNA vaccines, viral vector vaccines and plant-derived vaccines are found to be most effective against a number of bacterial and viral disorders

Oral vaccines: a novel approach

- 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**
- *'melt in the mouth'* vaccines that place under the tongue that delivers it into the blood stream.
- The most important example is the production of flu vaccine by *Bacillus* which melts in

Agricultura biotechnology

- It 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.

Bt Cotton

Biotechnology in Gene therapy

- Most, if not all, diseases have a genetic factor.
- For example, in disorders such as cystic fibrosis, haemophilia, and muscular dystrophy, changes in a gene directly result in the condition.
- 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

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.

Forms of gene therapy

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.

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.
- It is 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.

Biotechnology in Genetically Modified Organisms (GMOs

- These are living organisms whose genetic material has been artificially manipulated in a laboratory through genetic engineering.
- 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.

Transgenic Plants

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

Insect pest resistance

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. 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*

Improved nutritional qualities (biofortification)

- Transgenic plants have also been produced to provide functional food and nutraceuticals
- In many developing countries rice is the stapple food, but rice is deficient of many essential nutrients , malunion is a common problem.
- One of the common disease is the lack of vitamin-A.
- Vitamin –A is available in milk and vegetables like carrot.
- But , due to poverty many cannot afford this food
- 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).

- 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
- The genes for ferritin protein isolated from soybean and *Phaseolus* have

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

Modification in Post-harvest characteristics

- The problems faced by farmers are the method of storage of the products for long time without decaying the products.
- Most of these 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.

Plants as factories

Plants are used as bioreactor to produce many products 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

Example for biopharmaceuticals are:-

- 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.

Novel products

- 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.
- Transgenic plants, containing oil encoding gene from marine algae, produce oil that has nutritional value similar to cod- liver oil.
- Plant that will produce the antimalarial drug, Artemisinin.
- Genetically engineered opium poppy to produce more powerful painkillers.

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.

Tomatoes

Potatoes

Transgenic animals

Transgenic animals are animals animals such as mice, rats, rabbits, pig, sheep, cows, fowls, fish that have been produced through rDNA technology

Application of transgenic animals

- 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;

- 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
- developing animals specially created for use in xenografting.

Transgenic mice and cancer research

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 analyzed to find out if they lead to breast cancer in mice transformed with these genes

Transgenic farm animals

- 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

- 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

Transgenic cattle for food production

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

Transgenic cattle for human therapeutic production

Human proteins that have been expressed in milk include human lactoferrin, human alpha lactalbumin, human serum albumin and human bile salt stimulated lipase. 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. To make a human antibody product, the genetically modified cows are immunized with a vaccine containing the disease agent.

Transgenic Cow-Rosie

Transgenic Sheep

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

Transgenic pigs

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).

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.

Transgenic chicken

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.

Bioethics, bio-piracy and bio-patent

Bioethics

- Ethics usually deals with the matters related to socially acceptable moral duty, conduct and judgement.
- The introduction of Genetically Modified Organisms (GMOs) has led to a wider debate on bioethical concerns affecting social, economic and environmental spheres.
- Ethics in biotechnology also includes the general subject of what should and should not be done in using recombinant DNA techniques

Effects of Biotechnology on the Environment

Herbicide Use and Resistance

- One area of development involves adding the ability to produce pesticides and resistance to specific herbicides.
- These traits are helpful in food production. 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 protometal be of the herbicide.
- One such herbicide that has alternately been added is RoundUp.
- Crops of RoundUp ready soybeans have already been implemented into agricultural practices, possibly conferring RoundUp resistance to neighboring plants.

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.

Effects of Biotechnology on Human Health :

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 soya beans.
- The insertion of this gene inadvertently caused allergic reactions to the soya bean in those with known nut allergies (“Biotech Soybeans”).

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

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.

Food Additives

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

Biopatent and Biopiracy

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

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 government. Such a patent is considered morally unacceptable and fundamentally unequitable

Biopiracy

Most developed, industrialized and financially rich nations are poor in biodiversity or traditional knowledge whereas developing and underdeveloped nations have ample of biodiversity and they traditionally better know the use of their bio-resources. This leads to biopiracy

Examples of Biopiracy

Patenting of Neem (*Azadirachta indica*)

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

- 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 UPSTO has rejected all the claims

Basmati rice

Texmati Rice

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.

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THANK YOU