34. Biotechnology and its Application

1. Introduction

Biotechnology finds its application in medicine, therapeutics, diagnostics, bioremediation, genetically modified crops for agriculture, waste treatment, processed food & energy production. The thrust area of biotechnology includes:

- Improved organism mostly a microbe or pure enzyme acting as the best catalyst.
- Modifying the conditions to provide optimum condition through engineering so that catalyst acts to its optimum.
- Downstream processing technologies to purify the protein/organic compound.

This chapter deals with applications of biotechnology in agriculture (GM crops/GM food), in medicine (therapeutic agent, gene therapy), transgenic animals & their applications, Bioethics, Biopatent, and Biopiracy.

Biotechnology engages in research as a basis for scientific, technological and society innovation in industrial and environmental biotechnology. Within the department, scientists and engineers work together in disciplines ranging from genomics, metabolomics and biocatalysis to fermentation technology, environmental biotechnology, bioprocess technology and downstream processing.

2. Biotechnology Applications in Agriculture

Food production can be enhanced by

2.1 Agro-chemical based agriculture

Use of improved varieties of crops and use of agrochemicals (fertilizers and pesticides).

2.2 Organic agriculture

It relies on fertilizers of organic origin such as compost manure, green manure, Biopesticides and Biofertilisers. Promotes techniques such as crop rotation and companion planting. Biological pest control, mixed cropping and the fostering of insect predators are encouraged

2.3 Genetically engineered crop-based agriculture

Plants, bacteria, fungi and animals whose genes have been changed by manipulations are called Genetically Modified Organisms (GMOs).

Green revolution resulted in increasing the food supply almost three times. Green revolution is the great increase in the production of food grains (especially wheat & rice) that resulted in large part from the introduction of new, high yielding varieties beginning in the mid 20th century. Its early dramatic success was in Mexico & the Indian Subcontinent. The new varieties required large amount of chemical fertilizers & pesticides to produces high yield, raising concern about cost & potentially harmful environmental effects. This demands an alternate pathway that can result in maximum yield from the fields but the chemicals & fertilizer use is minimum i.e. harmful effects on the environment are reduced.

3. Transgenic Crop or Genetically Modified Crop

- Transgenic plants are the ones, whose DNA is modified using genetic engineering techniques. The aim is to introduce a new trait to the plant which does not occur naturally in the species.
- A transgenic plant contains a gene or genes that have been artificially inserted. The inserted gene sequence is known as the transgene, it may come from an unrelated plant or from a completely different species.
- This process provides advantages like improving shelf life, higher yield, improved quality, pest resistance, tolerant to heat, cold and drought resistance, against a variety of biotic and abiotic stresses.
- Transgenic plants can also be produced in such a way that they express foreign proteins with industrial and pharmaceutical value. Plants made up of vaccines or antibodies (Plantibodies) are especially striking as plants are free of human diseases, thus reducing screening costs for viruses and bacterial toxins.
- The first transgenic plants were reported in 1983. Since then, many recombinant proteins have been expressed in several important agronomic species of plants including tobacco, corn, tomato, potato, banana, alfalfa and canola. Tobacco plants were generally used, however potatoes and bananas are also considered, for the purpose of vaccines for human beings.
- A transgenic crop is a crop that contains and expresses a transgene. A popular term for transgenic crops is genetically modified crops or GM crops. The techniques used for the production of transgenic crops offer the following two unique advantages: any gene (from any organism or a gene synthesized chemically) can be used for transfer, and
- the change in genotype can be precisely controlled since only the transgene is added into the crop activities.

When a gene is introduced into the genome of an organisms, it can achieve one of the following.

3.1 Produce a protein of Interest

Hirudin : Is a protein that prevents blood clotting. The gene encoding hirudin was chemically synthesised. This gene was then transferred into *Brassica napus*, where hirudin accumulates in seeds. The hirudin is purified and used as medicine. In this case, the transgene product itself is the product of interest.

3.2 Produces a desired Phenotype

It produces a protein that on its own produces the desired phenotype. For example-a soil bacterium Bacillus thuringiensis produces a crystal (cry) protein that is toxic to larvae of certain insects.The gene encoding cry protein has been isolated and transferred into several crop. The crop expressing a cry gene is usually resistant to the group of insects for which the concerned cry protein is toxic. In such cases , the transgene product directly produces the phenotype of interest.

3.3 Modifies an existing biosynthetic pathway

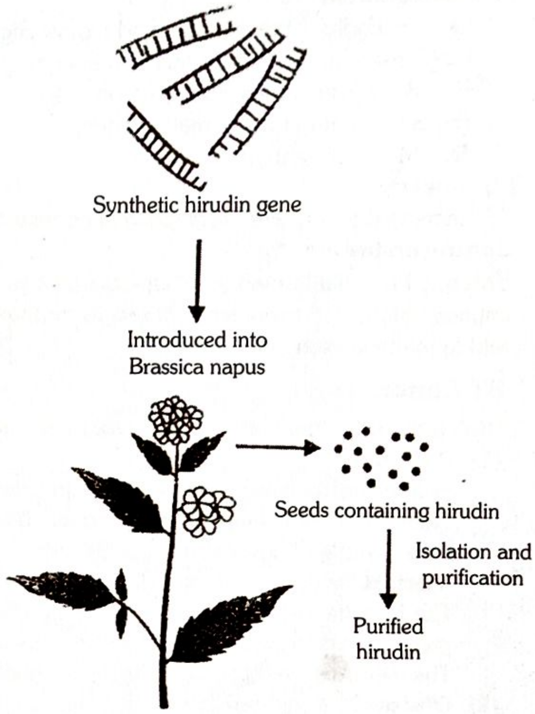
It modifies an existing biosynthetic pathway, so that a new end product is obtained. Transgenic rice and transgenic patato produce higher content of Vitamin-A and Protein Resp.

3.4 Masks Expression of a Native Gene

It prevents the expression of an existing native gene e.g. *Flavr savr* tomato

3.5 Flaver Savr Tomato

The tomato variety 'Flaver Savr' presents an example where expression of a native tomato gene has been blocked. Expression of a native gene can be stopped by many different methods. Fruit softening is promoted by the enzyme polygalacturonase, which degrades pectin. Production of polygalacturonase was blocked in the transgenic tomato variety 'Flavr Savr' Therefore, fruits of this tomato variety remain fresh and retain their flavour much longer than do the fruits of normal tomato varieties. In addition, the fruits have a superior taste and increased total soluble solids; these are unexpected bonus.



A simplified representation of the production of hirudin

4. Example of Genetically Modified Food Crop

The food prepared from the produce of genetically modified (transgenic) crops is called genetically modified food or, in short 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 transgene 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.

Important-

Biofortification is a method of breeding crops to increase their nutritional value. This can be done either through conventional selective breeding, or through genetic engineering, biofortification differs from ordinary fortification because it focuses on making plant foods more nutritious as the plants are growing, rather than having nutrients added to the foods when they are being processed. This is an improvement on ordinary fortification when it comes to providing nutrients for the rural poor, who rarely have access to commercially fortified foods. As such, biofortification is seen as an upcoming strategy for dealing with deficiencies of micronutrients in the developing world.

It has been argued that the above features of GM food could lead to the following problems when they are consumed.

- Firstly the transgene product may cause allergies, since it is a foreign protein.
- Finally, the bacteria present in the GM food. These bacteria would then become resistant to the concerned antibiotic. As a result, these bacteria could become difficult to manage.
- The scientist involved in the production of transgenic crops are addressing to these concerns. Efforts are being made to use other genes in place of antibiotic resistance genes. The toxic and allergenic actions of the transgene product can be adequately examined by detailed essays using suitable animal models.

4.1 GM Products : Benefits and Controversies

Benefits

(1) Crops

(i) Reduced taste and quality
(ii) Reduced maturation time
(iii) Increased nutrients, yields, and stress tolerance
(iv) Improved resistance to disease, pests and herbicides
(v) New products and growing techniques

(2) Animals

(i) Increased resistance, productivity, hardiness, and feed efficiency
(ii) Better yields of meat, eggs, and milk
(ii) Improved animal health and diagnostic methods

(3) Environment

(i) "Friendly" bioherbicides and bioinsecticides

(ii) Conservation of soil, water and energy

(iii) Bioprocessing for forestry products

(iv) Better natural waste management

(iv) More efficient processing

(4) Society

Increased food security for growing populations

Controversies

Safety : Potential human health impact: allergens, transfer of antibiotic resistance markers, unknown effects potential environmental impact: unintended transfer of transgenes through cross-pollination, unknown effects on other organisms (e.g. soil microbes), and flora and fauna biodiversity.

4.2 BT Cotton

DNA technology makes it possible to locate the genes that produce Bt proteins lethal to insects & transfer the gene into crop plants.

(1) Procedure

First scientists identify a strain of Bt that kills the targeted insect.

Then they isolate the gene that produces the lethal protein.

That gene is removed from the Bt bacterium & a gene conferring resistance to a chemical (usually antibiotic or herbicide) is attached that proves useful in later steps.

The Bt gene with the resistance gene-attached is inserted into plant cells. These modified or genetically transferred cells are then grown into complete plant by tissue culture.

The modified plant produces the same lethal protein as produced by the Bt bacteria because plants now have the same gene.

(2) Discovery and Study :

- B. thuringiensis was first discovered in 1902 by Japanese biologist Shigetane Ishwatari. In 1911, B. thuringiensis was rediscovered in Germany by Ernst Berliner, who isolated it as the cause of a disease called schlaffsucht in flour moth caterpillars. In 1976, Zakharyan reported the presence of a plasmid in a strain of B. thuringiensis and suggested the plasmid's involvement in endospore and crystal formation. B.thuringiensis is closely related to B. cereus, a soil bacterium, and B. anthracis, the cause of anthrax :
- The three organisms differ mainly in their plasmids. Like other members of the genus, all three are aerobes capable of producing endospores. Upon sporulation, B. thuringiensis forms crystals of proteinaceous insecticidal δ -endotoxins (called crystal proteins of cry proteins), which are encoded by cry genes. In most strains of B. thuringiensis the cry genes are located on the plasmid.
- Cry toxins have specific activities against insect species of the orders Lepidoptera (moths and butterflies), dipteral (flies and mosquitoes), coleopteran (beetles), hymenoptera (wasps, bees, ants and sawflies) and nematodes.
- Thus, B.thuringiensis serves as an important reservoir of cry toxins for production of biologically insecticides and insect-resistant genetically modified crops. When insects ingest toxin crystals, the alkaline pH of their digestive tract activates the toxin. Cry inserts into the insect gut cell membrane, forming a pore. The pore results cell lysis and eventual death of the insect.
- B.thuringiensis forms protein crystals 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 protoxin but once an insect ingests the inactive toxin, it is converted into an active form of toxin due to the alkaline pH of the gut which 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. Bt is not harmful to humans, other mammals, birds fish or beneficial insects.
- 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 cry/Ac and cry// Ab control the cotton bollworms, that of cry / Ab.

Although Bt genes have been introduced into tobacco, tomatoes, cotton and other broadleaf plants, gene transfer technology for orn is a recent achievement. The development of corn plants expression Bt proteins requires substantial changes in the Bt genes, including the creation of synthetic versions of the genes, rather than the microbial Bt gene itself.

Important

There are several advantages in expressing Bt toxins in transgenic Bt crops.

(1) The level of toxin expression can be very high thus delivering sufficient dosage to the pest.

(2) The toxin expression is contained within the plant system and hence only those insects that feed on the crop perish.

(3) The toxin expression can be modulated by using tissue-specific promoters, and replaces the use of synthetic pesticides in the environment. The latter observation has been well documented worldwide.

4.3 Pest Resistant Plant

Root-knot nematodes are the most economically important group of plant-parasitic nematodes worldwide. They attack nearly every food and fiber crop grown, about 2,000 plant species in all. The nematode invades plant roots, and by feeding on the roots, cells, they cause the roots to grow large galls, or knots, damaging the crop and reducing its yields.

- The most cost-effective and sustainable management tactic for preventing root-knot nematode damage and reducing growers losses is to develop resistant plants that prevent the nematode from feeding on the roots. Because root-knot nematode resistance doesn't come naturally in most crops, bioengineering is required.
- Four common root-knot nematode species (mainly meloidogyne incognita) account for 95 percent of all infestations in agricultural land. By discovering a root-knot nematode parasitism gene that's essential for the nematode to infect crops, the scientists have developed a resistance gene effective against all four species.
- **Using a technique called RNA interference (RNA), the researchers have effectively tuned the nematode's biology against itself**. They genetically modified Arabidopsis, a model plant, to produce double-stranded RNA (dsRNA) to knock out the specific parasitism gene in the nematode when it feeds on the plant roots.

Figure :– 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

Important

Long double-strand RNAs (dsRNAs; typically>200nt) can be used to silence the expression of target genes in a variety of organisms and cell types (e.g. worms, fruit flies and plants).

Upon introduction, the long dsRNAs enter a cellular pathway that is commonly referred to as the RNA interference (RNA) pathway.

First, the dsRNA get processed into 20-25 nucleotide (nt) small interfering RNAs (siRNAs) by an RNase III-like enzyme called Dicer (initiation step).

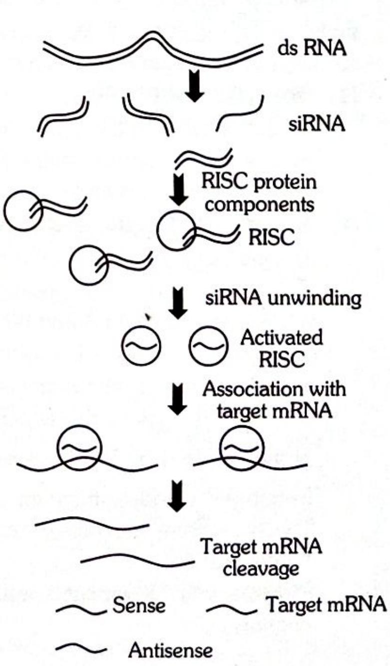
Then the complexes (RISCs), unwinding in the process. The siRNA strand subsequently guides the RISCs to complementary RNA molecules, where they cleave and destroy the cognate RNA (effecter step). Cleavage of cognate RNA takes place near the middle of the region bound by the siRNA strand.

In mammalian cells, introduction of long dsRNA (>30 nt) initiates a potent antiviral response, exemplified by nonspecific inhibition of protein synthesis and RNA degradation. The mammalian antiviral response can be bypassed, however, by the introduction or expr3ession of siRNAs.

4.4 The Mechanism of RNA Interference (RNA)

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 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 is produced both sense and anti-sense RNA in the host cells. These two RNAs being complementary to each other formed a double stranded RNA 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.
- "The knocked out the parasitism gene has this effective range of root-knot nematode resistance."
- The efforts have been directed primarily at understanding the molecular tools the nematode uses to infect plants.
- This is a prerequisite for bioengineering durable resistance to these nematodes in crop plants.

4.5 Golden Rice

- **Golden rice** is a variety of rice (*Oryza sativa*) produced through genetic engineering to biosynthesize beta-carotene, a precursor of vitamin A, in the edible parts of rice.
- It is intended to produce a fortified food to be grown and consumed in areas with a shortage of dietary vitamin A, a deficiency which is estimated to kill 670,000 children under the age of 5 each year.
- Rice is a staple food crop for over half of the world's population, making up 30–72% of the energy intake for people in Asian countries, making it the perfect crop for targeting vitamin deficiencies.
- Golden rice differs from its parcntal strain by the addition of three beta-carotene biosynthesis genes. The parental strain can naturally produce beta-carotene in its leaves, where it is involved in photosynthesis.
- However, the plant does not normally produce the pigment in the endosperm, where photosynthesis does not occur. Golden rice has met significant opposition from environmental and anti-globalization activists that claim that there are sustainable, long-lasting and more efficient ways to solve vitamin A deficiency that do not compromise food, nutrition and financial security.
- In 2005, Golden Rice 2 was announced, which produces up to 23 times more beta-carotene than the original golden rice. To receive the USDA's Recommended Dietary Allowance (RDA), it is estimated that 144 g of the high-yielding strain would have to be eaten. Bio availability of the carotene from golden rice has been confirmed and found to be an effective source of vitamin A for humans.

5. Biotechnological Application in Medicine

5.1 Therapeutic Agents

Proteins with potential as pharmaceutical agents are produced agents are produced by using genetically engineered organism. Enzymes have also been used for this purpose, e.g., DNase and alginate lyase have been used in aerosols.

Some known Examples are Given Below:

(i) Human growth hormone obtained from E.coli is used for treatment of dwarfness.

(ii) Chorionic gonadotropin hormone produced by genetic engineering is used for treatment of infertility.

(iii) Interferons produced by E.coli are commercially used for treatment of viral infections and cancer. Interferons were first obtained through DNA recombinant technique by Charles weisman in 1980. He inserted the gene for interferon production in E.coli.

(iv) Interleukins produced by E.coli are used for stimulating immunity system.

(v) Tissue plasminogen Activator (TPA)- an enzyme is used for dissolving blood clot after heart attack and stroke.

(vi) Antihemophilic human factor VIII is used by people with hemophilia to prevent and control bleeding or to prepare them for surgery.

(vii) Platelet derived growth factor produced by recombinant DNA technology is useful for stimulating wound healing.

(viii) Penicillin G acylase is also produced by genetic engineering. This enzyme is used for converting penicillin into 6-amino penicillin acid for the formation of new antibiotics.

5.2 Genetically Engineered Insulin

Since the discovery of insulin by Banting and best (1921), and is use the treatment of diabetes, it was derived from pancreatic glands of abattoir animals. This hormone, produced and secreted by the beta cells of the pancreas's islets of Langerhans, regulates the use and storage of food, particularly carbohydrates. Although bovine and porcine insulin is similar to human insulin, their composition is slightly different. It therefore, causes adverse effects due to regular injection, this being a foreign substance. This observation led to the synthesis of human insulin which is chemically identical to its naturally produced component.

(1) Structure of Insulin

Insulin consists of 51 amino acids forming two short polypeptide chains-chain A having 21 amino acids and chain B with 30 amino acids. The two chains are linked by disulfide bond. In animals, including humans, insulin occurs as proinsulin. It is made of chain A, chain B and chain C (30 amino acids). As the insulin matures, chain C is removed.

(2) Genetically Engineered Insulin

The genetic engineering of insulin begins with identification and separation of DNA sequences coding for chain A and chain B. This was found to be present at the top of the short arm of the eleventh chromosome. It contains 153 nucleotidesw-63 nucleotides for chain A and 90 nucleotides for chain B.

These sequences were introduced into plasmid (pBR322) of Escherichia coli-common human colon bacterium. It is said to be the factory used in genetic engineering of insulin.

In E.coli, $\beta-$galactosidase controls the transcription of these genes, therefore insulin gene needs to be tied to this enzyme. The protein formed by E.coli consists partly of $\beta-$galactosidase joined to either A or B chain of insulin These are then extracted from b-galactosidase fragment and purified.

The two chains are mixed and reconnected in a reaction that forms disulfide bridges resulting in pure humulin-the synthetic human insulin.

Proteins with Therapeutic and industrial value that have been produced (but not commercialized) in the milk of Transgenic animals.

Protein	Animal	use
Antithrombin III	Goat	Reduce the amount of blood needed in some surgeries
Factor VIII, Factor IX	Got, Pig, Sheep	Treatment of hemophilia
CFTR	Sheep	Treatment of cystic fibrosis
Alpha-1 antitrypsin	Sheep	Treatment of emphysema
Lysostaphin	Cow	An anti-bacterial compound that prevents mastitis in cows
Spider silk protein	Goat	Production of ultra-strong, lightweight medical and industrial materials

6. Gene Therapy

Much attention has been focused on the so-called genetic metabolic disease in which a defective gene causes an enzyme to be either absent or ineffective in catalyzing a particular metabolic reaction effectively. A potential approach to the treatment of genetic disorders in man in is gene therapy.

6.1 Definition

This is a technique whereby the absent or faulty gene is replaced by a working gene, so that the body can make the correct enzyme or protein and consequently eliminate the root cause of the disease.

6.2 History

The first clinical gene therapy was given in 1990 to a 4-year old girl with adenosine (ADA) deficiency. This enzyme is crucial for the immune system to function.

- The disorder is caused due to the deletion of the gene for adenosine deaminase.
- In some children, ADA deficiency can be cured by bone marrow transplantation; in others, it can be treated by enzyme replacement therapy, in which functional ADA is given to the patient by injection.
- But the problem with both of these approaches is that they are not completely curative.
- As a first step towards gene therapy, lymphocytes from the blood of the patient are grown in a culture outside the body.
- A functional ADA cDNA (using a retroviral vector) is the introduced into these lymphocytes, which are subsequently returned to the patient.
- However as these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes However, if the gene isolated from marrow cells producing ADA is introduced into cells at early embryonic stages, it could be a permanent cure.
- Genetic engineering research has produced a powerful tool for pinopointing specific diseases rapidly and accurately. Short pieces of DNA called DNA probes can be designed to stick very specifically to certain other pieces of DNA.

The technique relies upon the fact that complementary pieces of DNA stick together. DNA probes are more specific and have the potential to be more sensitive than conventional diagnostic methods, and it should be possible in the near future to distinguish between defective genes and their normal counterparts, an important development.

7. Molecular Diagnosis

For effective treatment of a disease, early diagnosis and understanding, its pathophysiology is very important. Using conventional methods of diagnosis (serum and urine analysis, etc), early detection is not possible. Recombinant DNA technology, Polymerase chain reaction (PCR) and enzyme linked immune-sorbent assay (ELISA) are some of the techniques that serve the purpose of early diagnosis.

7.1 PCR (Polymerase Chain Reaction)

Presence of a pathogen (bacteria viruses, etc) is normally suspected only when the pathogen has produced a disease symptom. By this time concentration of pathogen is already very high in the body. However, very low concentration of a bacteria or virus (at a time when the symptoms of the disease are not yet visible) can be detected by amplification of their nucleic acid by PCR, which is now routinely used to detect HIV in suspected AIDS patients. It is being used to detect mutations in genes in suspected cancer patients too. It is a powerful technique to identify many other genetic disorders.

7.2 Southern Blotting

- DNA is usually isolated from white blood cells & has to be cut into smaller pieces to be analysed. This is accomplished by restriction enzymes. Eco RI (a restriction enzyme from E.coli) will cut DNA wherever the sequence GAATTC appears. Exposure to this enzyme results in the DNA being chopped into millions of fragments of varying size, called restriction fragments.
- Once cut, the DNA is loaded into a well on one end of a slab of gel. The fragments are then separated according to size by electrophoresis. As electric current passes through the gel, the fragments move according to size. The bigger fragments stay close to the origin, and the smaller fragments move farther down the length of the gel. The DNA is the denatured (by exposure to alkaline solutions) to render the DNA single-stranded (instead of the natural double-stranded from).
- Since the gel is difficult to handle, the DNA is transferred to a nitro cellulose paper to create a southern blot (named after the researcher who developed the procedure).
- The DNA probe which is radioactively labelled (or fluorescently labelled) is then applied to the southern blot. Since the probe is also single-stranded, it will seek the single-stranded DNA fragments that are complementary, and undergo hybridization.

- The excess probe is washed out and only the bound prove will remain on the southern blot paper. This is then laid on an X-ray film. The radioactive probe will leave bands on the X-ray film.
- Depending on the type of probe used, there could be hundreds of bands (much like bar codes) or only a few bands present on the X-ray film. By having several wells on the end of the gel, several samples can be loaded, and DNA fragments in corresponding lanes can be analyzed concurrently. By running control samples, with known DNA fragments sizes, on the same gel with patient samples, it is possible to identify changes in the size of a DNA fragment and, therefore, a change in a specific gene. Since each step takes about a day and since samples are batched, the procedure ordinarily takes on to two weeks to complete.

8. Transgenic Animals

There are various definitions for the term transgenic animal. A transgenic animal is one whose genome has been changed to carry genes from other species. The nucleus of all cells in every living organism contains genes made up of DNA. These genes store information that regulates how our bodies form and function. Genes can be altered artificially, so that some characteristic of an animal are changed. For example, an embryo can have an extra, functioning of another source artificially introduced into it, or a gene introduced which can knock out the functioning of another particular gene in the embryo. Animals that have their DNA manipulated in this way are known as transgenic animals. The majority of transgenic animals produced so far are mice, the animal that pioneered the technology. The first successful transgenic animal was a mouse. A few later, it was followed by rabbits. Pigs, sheep, and cattle.

8.1 How are Transgenic Animals Produced

To date, there are three basic method of producing transgenic animals:

(i) DNA microinjection.
(ii) Retrovirus-mediated gene transfer.
(iii) Embryonic stem cell-mediated gene transfer.

Gene transfer by microinjection is the predominant method used to produce transgenic farm animals. Since the insertion of DNA results in a random process, transgenic are mated to ensure that their offspring acquire the desired transgene. However, the success rate of producing transgenic animals individually by these methods is very low and it may be more efficient to use cloning techniques to increases their numbers. For example, gene transfer studies revealed that only 0.6% of transgenic pigs were born with a desired gene after 7,000 eggs were injected with a specific transgene.

8.2 How do Transgenic Animals Contribute to Human Welfare

The benefits of these animals to human can be grouped into following areas:

(1) Agriculture
(2) Medicine
(3) Industry

The examples below are not intended to be complete but only to provide a sampling to the benefits

8.3 Agriculture Applications

(1) **Breeding :** Farmers have always used selective breeding to produce animals that exhibit desired traits (e.g., increased milk production, high growth rate). Traditional breeding is a time consuming, difficult task, when technology using molecular biology was developed, it became possible to develop traits in animals in a shorter time and with more precision. In addition, if offers the farmer an easy way to increase yields.

(2) **Quantity :** Transgenic cows exist that produce more milk or with less lactose or cholesterol, pigs and cattle that have more meat on them, and sheep that grow more wool. In the past, termers used growth hormones to spur the development of animals but this technique was problematic, especially since residue of the hormones remained in the animal product.

(3) **Disease Resistance :** Scientists are attempting to produce disease-resistant animals, such as influenza-resistance pigs, but a very limited number of genes are currently known to be responsible for resistance to disease in farm animals.

8.4 Medical Applications

(1) **Xenotransplantation :** patients die every year for lack of a replacement heart, liver kidney. For example, about 5,000 organs are needed each year in the united kingdom alone. Transgenic pigs may provide the transplant organs needed to alleviate the shortfall. Currently, xenotransplantation is hampered by a pig protein that can cause donor rejection but research is underway to remove the pig protein and replace it with a human protein.

(2) **Nutritional Supplements and Pharmaceuticals :** Products such as insulin, growth hormone, and blood anti-clotting factors may soon be or have already been obtained from the milk of transgenic cows, sheep or goats. Research is also underway to manufacture milk through transgenic for treatment of debilitating disease such as phenylketonuria (PKU), hereditary emphysema, and cystic fibrosis.

In 1997, the first transgenic cow, rosie, produced human protein-enriched milk at 2.4 grams perlitre. This transgenic milk is a more nutritionally balanced product than natural bovine milk and could be given to babies or the elderly with special nutritional or digestive needs. Rosie's milk contains the human gene alpha-lactalbumin.

(3) **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 to the polio vaccine. If successful and found to be reliable, they could replace the use of monkeys to test the safety of batches of eh vaccine.

8.5 Industrial Application

(1) In 2001, two scientists at Nexia Biotechnologies in Canada spider genes into the cells of lactating goats. The goats began to manufacture silk along with their milk and secrete tiny silk strands from their body by the bucketful. By extracting polymer strands

- 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 bioresources include all those organisms that can be used to derive commercial benefits: Traditional knowledge related to bioresources is the knowledge developed by various communities over long history,
- regarding the utilisation of the bioresources, e.g. use of herbs, etc. as drugs, often, this traditional knowledge can be exploited to develop modern commercial processes. The traditional knowledge suggests the direction to be followed, and saves considerable time, effort and expenditure for their commercialisation.

Important

Intellectual property (IP) is a term referring to a number of distinct types of creations of the mind for which a set of exclusive rights are recognized-and the corresponding fields of law under intellectual property law, owners are granted certain exclusive rights to a variety of intangible assets, such as musical, literary, and artistic works, discoveries and inventions; and words, phrases, symbols and designs. Common types of intellectual property include copyrights, trademarks, patents, industrial designed rights and trade secrets in some jurisdictions.

10.3 Institutions and companies of industrialized nations are collecting and exploiting the bioresources, as follows.

(i) They are collecting and patenting the genetic resources themselves. For examples, a patent granted in U.S.A. covers the entire 'basmati' rice germplasm indigenous to our country.

(ii) The bioresources are being analysed for identification of valuable biomolecules. A biomolecules 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 bioresources and patented. These genes are then used to generate commercial products.

(iv) The traditional knowledge related to bioresources is utilized to achieve the above objectives. In some cases, the traditional knowledge itself may be the subject of a patent.

- A west African plant, pentadiplandra brazzeana products a protein called brazzein, which approximately 2,000 times as sweet as sugar. In addition, brazzein is a low-calorie sweetener. Local people in U.S.A. Subsequently, the gene encoding brazzein was also isolated, sequenced and patented in U.S.A. It is proposed to transfer the brazzein gene into maize and express it in maize kernels. These kernels will then be used for the extraction of brazzein. This development could have serious implications for countries exporting large quantities of sugar.
- Bioresources of the developing world have always been commercially exploited by the industrialized. Nations without an adequate compensation to the developing world. Exploitation has dramatically increased in pace with the development of powerful analytical tools and techniques. There has been a growing realization of this injustice and demands are being made for adequate compensation and benefit sharing. Some nations are developing comprehensive laws to prevent unauthorised exploitation of their bioresources 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.

34. Biotechnology and its Applications – Multiple Choice Questions

1. Biotechnology and its Application

1. Utility of fungi for steroid conversion was demonstrated by
(a) Pasteur and Jaubert (b) Kohler and Milstein
(c) Murray and Peterson (d) Waksman and Woodruff

2. Most widely used bioweapon is
(a) *Bacillus subtilis* (b) *Pseudomonas putida*
(c) *Bacillus anthracis* (d) None of the above

3. The vaccine of Hepatitis-B is a
(a) First generation vaccine
(b) Interferon
(c) Second generation vaccine
(d) Third generation vaccine

4. Isolation of ***Bt*** gene from bacterium (*Bacillus thuringiensis*) was taken up in the year
(a) 1977 (b) 1980
(c) 1997 (d) 1990

5. Crown gall disease in plants is caused by
(a) Ti-plasmid (b) Pi-plasmid
(c) Bacteria (d) Virus

6. GEAC stands for
(a) Genome Engineering Action Committee
(b) Ground Environment Action Committee
(c) Genetic Engineering Approval Committee
(d) Genetic Engineering Approval Committee

7. C-peptide of human insulin is
(a) A part of mature insulin molecule
(b) Responsible for formation of disulphide bridges
(c) Removed during maturation of proinsulin to insulin
(d) Responsible for its biological activity

8. Bt cotton is not
(a) A GM plant
(b) Insect resistant
(c) A bacterial gene expressing system
(d) Resistant to all pesticides

9. $\alpha-1$ antitrypsin is
(a) An antacid (b) An enzyme
(c) Used to treat arthritis
(d) Used to treat emphysema

10. The site of production of ADA in the body is
(a) Erythrocytes (b) Lymphocytes
(c) Blood plasma (d) Osteocytes

11. Choose the correct option regarding Retrovirus
(a) An RNA virus that synthesizes DNA during infection
(b) A DNA virus that synthesized RNA during infection
(c) A ssDNA virus
(d) A dsRNA virus

12. A protoxin is
(a) A primitive toxin
(b) A denatured toxin
(c) Toxin produced by protozoa
(d) Inactive toxin

13. Pathophysiology is the
(a) Study of physiology of pathogen
(b) Study of normal physiology of host
(c) Study of altered physiology of host
(d) None of the above

14. The first clinical gene therapy was done for the treatment of
(a) AIDS
(b) Cancer
(c) Cystic fibrosis
(d) SCID (Severe Combined Immunodeficiency resulting from deficiency of ADA)

15. Silencing of a gene could be achieved through the use of
(a) RNA only
(b) Antisense RNA only
(c) Both RNA and antisense RNA
(d) None of the above

16. Genetically engineered bacteria are being employed for production of
(a) Thyroxine (b) Human insulin
(c) Cortisol (d) Epinephrine

17. 'Gray biotechnology' is referred to
(a) Medical process (b) Industrial process
(c) Agricultural process (d) Aquatic process

18. The new strain of bacteria produced by biotechnology in alcohol industry is
(a) *Escherichia coli*
(b) *Saccharomyces cerevisiae*
(c) *Bacillus subtilis*
(d) *Pseudomonas putida*

19. Find the incorrect statement
(a) Gene therapy is a genetic engineering technique used to treat diseases at molecular level by replacing defective genes with normal genes
(b) Calcitonin is a medically useful recombinant product in the treatment of infertility
(c) Bt toxin is a biodegradable insecticide obtained from *Bacillus thuringiensis*
(d) *Trichoderma* species is a biocontrol agent for fungal diseases of plants
(e) Totipotency is the potential ability of a cell to develop into a complete plant

20. Choose the correct statement with reference to "Dolly"
(a) She was created by taking nucleus from unfertilised egg.
(b) She was created by taking nucleus from udder cell and cytoplasm from unfertilised egg
(c) She was created by taking cytoplasm from udder cells and nucleus from fertilised egg
(d) She was created in the test tube

21. Match List I with List II and select the correct option

List I		List II	
A.	*Bacillus thuringiensis*	1.	Production of chitinases
B.	*Rhizobium meliloti*	2.	Scavenging of oil spills
C.	*Escherichia coli*	3.	Incorporation of 'nif' gene
D.	*Pseudomonas putida*	4.	Production of Bt toxin
E.	*Trichoderma*	5.	Production of human insulin

(a) A – 2, B – 4, C – 1, D – 5, E – 3
(b) A – 2, B – 4, C – 5, D – 1, E – 3
(c) A – 4, B – 3, C – 5, D – 2, E – 1
(d) A – 3, B – 4, C – 5, D – 1, E – 2
(e) A – 4, B – 2, C – 5, D – 3, E – 1

22. The enzyme is TPA is used to
(a) Maintain turgor pressure
(b) Strengthen tissues
(c) Increase plasma
(d) Dissolve blood clots

2. NEET

1. Human insulin is being commercially produced from a transgenic species of **[2008]**
(a) *Rhizobium* (b) *Saccharomyces*
(c) Escherichia (d) *Mycobacterium*

2. Kohler and Milstein developed biotechnology for the production of **[1989; 2002]**
(a) Myelomas (b) Steroid conversion
(c) Monoclonal antibodies (d) Immobilised enzymes

3. What is true about Bt toxin **[2009; 2015]**
(a) The inactive protoxin gets converted into active form in the insect gut
(b) Bt protein exists as active toxin in the Bacillus
(c) The activated toxin enters the ovaries of the pest to sterilize it and thus prevent its multiplication
(d) The concerned Bacillus has antitoxins

4. Tobacco plants resistant to a nematode have been developed by the introduction of DNA that produced (in the host cells) **[2012]**
(a) Both sense and antisense RNA
(b) A particular hormone
(c) An antifeedant
(d) A toxic protein

5. First cloned animal is **[2000]**
(a) Dog (b) Molly
(c) Dolly sheep (d) Polly sheep

6. Which part of the tobacco plant is infected by *Meloidogyne incognita* **[2016]**
(a) Flower (b) Leaf
(c) Stem (d) Root

7. Which of the following Bt crops is being grown in India by the farmers **[2013]**
(a) Soyabean (b) Maize
(c) Cotton (d) Brinjal

8. Which of the following represents the action of insulin **[2013]**
(a) Increases blood glucose levels by stimulating glucagon production
(b) Decreases blood glucose levels by forming glycogen
(c) Increases blood glucose level by promoting cellular uptake of glucose
(d) Increases blood glucose levels by hydrolysis of glycogen

9. Which one of the following vectors is used to replace the defective gene in gene therapy **[2013]**
(a) Adenovirus (b) Cosmid
(c) Ri plasmid (d) Ti plasmid

10. Golden rice is a promising transgenic crop. When released for cultivation, it will help in **[2006; 2015]**
(a) Herbicide tolerance
(b) Producing a petrol-like fuel from rice
(c) Alleviation of vitamin A deficiency
(d) Pest resistance

11. Which of the following correctly defines a transgenic animal **[1995]**
(a) An animal which has foreign DNA and RNA in some of its cells because of an injection of DNA and RNA into the nucleus of the zygote from which it is developed
(b) An animal which has foreign DNA in all its cells because of an injection of DNA into the nucleus of the zygote from which it is developed
(c) An animal which has foreign DNA in some of its cells because of an injection of DNA into the nuclei of some of the cells of the blastocyst
(d) An animal which has foreign DNA in all its cells because of an injection of DNA into the nuclei of some of the cells in adulthood

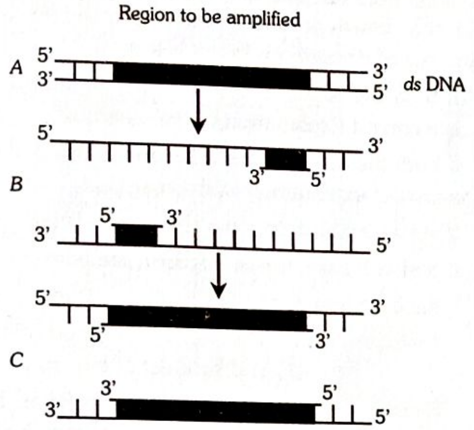
12. Maximum number of existing transgenic animals is of **[2011]**

Or

Transgenic rates, rabbits, pigs, sheep, cows and fish have produced although over 95% of all existing transgenic animals are
(a) Pig (b) Fish
(c) Mice (d) Cow

13. What is antisense technology [2008]
(a) When a piece of RNA that is complementary in sequence is used to stop expression of a specific gene
(b) RNA polymerase producing DNA
(c) A cell displaying a foreion antigen used for synthesis of antigens
(d) Production of somaclonal variants in tissue cultures

14. The figure below shows three steps (A, B, C) of Polymerase Chain Reaction (PCR). Select the option giving correct identification together with what it represents



Options [2012]
(a) B – Denaturation at a temperature of about 98°C separating the two DNA strands
(b) A – Denaturation at a temperature of about 50°C
(c) C – Extension in the presence of heat stable DNA polymerase
(d) A – Annealing with two sets of primers

15. The bacterium bacillus thuriengiensis is widely used in contemporary biology as [2009]
(a) Source of industrial enzyme
(b) Indicator of water pollution
(c) Insecticide
(d) Agent for production of dairy products

16. Transgenic plants are the ones [2009]
(a) Grown in artificial medium after hybridization in the field
(b) Produced by a somatic embryo in artificial medium
(c) Generated by introducing foreign *DNA* into a cell and regenerating a plant from that cell
(d) Produced after prolonged fusion in artificial medium.

17. An improved variety of transgenic basmati rice [2010]
(a) Does not require chemical fertilizers and growth hormones
(b) Gives high yield and is rich in vitamin *A*
(c) Is completely resistant to all insect pests and diseases of paddy
(d) Gives high yield but has no characteristic aroma.

18. Which body of the government of India regulate *GM* research and safety of introducing *GM* organisms for public services [2015]
(a) Genetic Engineering Approval Committee
(b) Research committee on Genetic Manipulation
(c) Bio safety committee
(d) Indian council of Agricultural Research.

19. Select the correct match [2018]
(a) Francois Jacob and Jacques Monod – Lac operon
(b) Matthew Meselson and F. Stahl – Pisum sativum
(c) Alfred Hershey and Martha Chase – TMV
(d) Alec Jeffreys – Streptococcus pneumoniae

20. The experimental proof for semiconservative replication of DNA was first shown in a [2018]
(a) Virus (b) Plant
(c) Bacterium (d) Fungus

21. A 'new' variety of rice was patented by a foreign company, though such varieties have been present in India for a long time. This is related to [2018]
(a) Basmati (b) Lerma Rojo
(c) Sharbati Sonora (d) Co-667

22. Which of the following is commonly used as a vector for introducing a DNA fragment in human lymphocytes [2018]
(a) pBR 322 (b) λ phage
(c) Ti plasmid (d) Retrovirus

23. In India, the organisation responsible for assessing the safety of introducing genetically modified organisms for public use is [2018]
(a) Genetic Engineering Appraisal Committee (GEAC)
(b) Research Committee on Genetic Manipulation (RCGM)
(c) Council for Scientific and Industrial Research (CSIR)
(d) Indian Council of Medical Research (ICMR)

3. AIIMS

1. Monoclonal antibodies are [2010]
(a) Obtained from a cell and act on one antigen
(b) Obtained from a group of cells and act on more than one antigens
(c) Obtained from a group of same type of cells and act on single antigen
(d) Obtained from a group of same type of cells and act on more than one antigens

2. An example of gene therapy is [2004]
(a) Production of injectable Hepatitis *B* vaccine
(b) Production of vaccines in food crops like potatoes which can be eaten
(c) Introduction of gene for adenosine deaminase in persons suffering from Severe Combined Immunodeficiency (SCID)
(d) Production of test tube babies by artificial insemination and implantation of fertilized eggs

3. A tumour inducing plasmid widely used in the production of transgenic plants is that of **[2005]**
(a) *Escherichia coli*
(b) *Bacillus thuringiensis*
(c) *Staphylococcus aureus*
(d) *Agrobacterium tumefaciens*

4. Hybridomas are employed for **[1986]**
(a) Synthesis of antibiotics
(b) Killing cancer cells
(c) Synthesis of monoclonal (somaclonal) antibodies
(d) Production of somatic hybrids

5. This method of finding a gene is used when researchers know very little about the gene they are trying to find. This process results in a complete gene library : a collection of copies of DNA fragments that represent the entire genome of an organism **[2009]**
(a) Cloning (b) Shotgun cloning
(c) Gene synthesis cloning (d) PCR

6. Hybridomas are the result of fusion of **[2007]**
(a) Male reproductive cells
(b) Female reproductive cells
(c) Normal antibody producing cells with myeloma
(d) Abnormal antibody producing cells with myeloma

7. Which one of the following is a correct statement **[2005]**
(a) "Bt" in "Bt-cotton" indicates that it is a genetically modified organism produced through biotechnology
(b) Somatic hybridization involves fusion of two complete plant cells carrying desired genes
(c) The anticoagulant hirudin is being produced from transgenic *Brassica napus* seeds
(d) "Flavr Savr" variety of tomato has enhanced the production of ethylene which improves its taste

8. Genetically engineered bovine (bST), sometimes called rbST (recombinant bovine somatotropin) or rbGH (recombinant bovine growth hormone) are used in the **[2009]**
(a) Therapeutic drugs (b) Agriculture
(c) Dairy industry (d) DNA fingerprinting

9. RNA interference is essential for the **[2012]**
(a) Cell proliferation (b) Cell defence
(c) Cell differentiation (d) Micropropagation

10. Cultivation of *Bt* cotton has been much in the news. The prefix *Bt* means **[2008]**
(a) Barium treated cotton seeds
(b) Bigger thread variety of cotton with better tensile strength
(c) Produces by biotechnology using restriction enzymes and ligases
(d) Carrying an endotoxin gene from Bacillus thuriengiensis

4. Assertion and Reason

Read the assertion and reason carefully to mark the correct option out of the options given below :
(a) If both the assertion and the reason are true and the reason is a correct explanation of the assertion
(b) If both the assertion and reason are true but the reason is not a correct explanation of the assertion
(c) If the assertion is true but the reason is false
(d) If both the assertion and reason are false
(e) If the assertion is false but reason is true

1. Assertion : The transgenic food may cause toxicity and product allergy in human beings.
Reason : The bacteria present in alimentary canal of human beings may become resistant to the antibiotics by taking up the antibiotic resistant gene that is present in the GM food.

2. Assertion : *Agrobacterium tumefaciens* is popular in genetic engineering because this bacterium is associated with the roots of all cereal and pulse crops.
Reason : A gene incorporated in the bacterial chromosomal genome gets automatically transferred to the crop with which the bacterium is associated.]

3. Assertion : The uptake of DNA during transformation is an active, energy requiring process.
Reason : Transformation occurs in only those bacteria, which possess the enzymatic machinery involved in the active uptake and recombination.