Chaudhary Mahadeo Prasad College

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Advanced Cytogenetics

Unit V: Genetic Engineering and its applications

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CONCEPT MAP OF

GENETIC ENGINEERING

Collection of techniques that alter the genetic constitution of cells or individuals

INVOLVES FOUR BASIC STEPS OF RDT / MOLECULAR CLONING

1. Isolation of DNA fragments

2. Joining the DNA fragments to a suitable vector

3. Introduction of the vector into a host cell

4. Selection of colonies with desired sequence

GENERATION OF GENETICALLY MODIFIED ORGANISMS (GMOS)

Has Applications in

Basic Research

Food Industry

Crop and Animal trait improvement

Environmental Restoration Pharmaceutical and Health

But raises some Legal and Ethical Questions

CONCEPT OF GENETIC ENGINEERING

By definition, genetic engineering is the direct alteration of an organism's genome which is achieved through manipulation of the DNA. This is achieved by using “recombinant DNA technology” which involves different techniques to insert, alter, or cut out pieces of DNA that contain one or more genes of interest. This is also known as genetic modification, gene transfer or transgenesis. Doing this is possible because DNA is like a universal language; all DNA for all organisms is made up of the same nucleotide building blocks. Thus, it is possible for genes from one organism to be read by another organism. Main focus of genetic engineering is:

- Gene isolation,
- Gene modification so that they can be transferred into and function within a new organism of a different species (transgenics) or the same species (cisgenics),
- Gene removal, and
- Evaluating the success of resultant gene combinations.

In practice, since DNA contains the genes to build certain proteins, by changing the DNA sequence, attempts are on to design a new gene for a cell/organism resulting in a different protein and also making a cell capable of performing the desired functions. The resultant organism is broadly referred as genetically modified organism (GMO).

The final aim of genetic engineering in higher eukaryotes results in two broad classes of GMOs which are:

• Genetically Modified Plants / Animals are designed for expression of the cloned genes for basic research on gene expression or for the production of useful proteins in tissue culture.

• Transgenic Plants / Animals are designed as a result of alteration of the genetic makeup of the organism in which all the cells will carry the genetic modification (shown in fig. 1.)

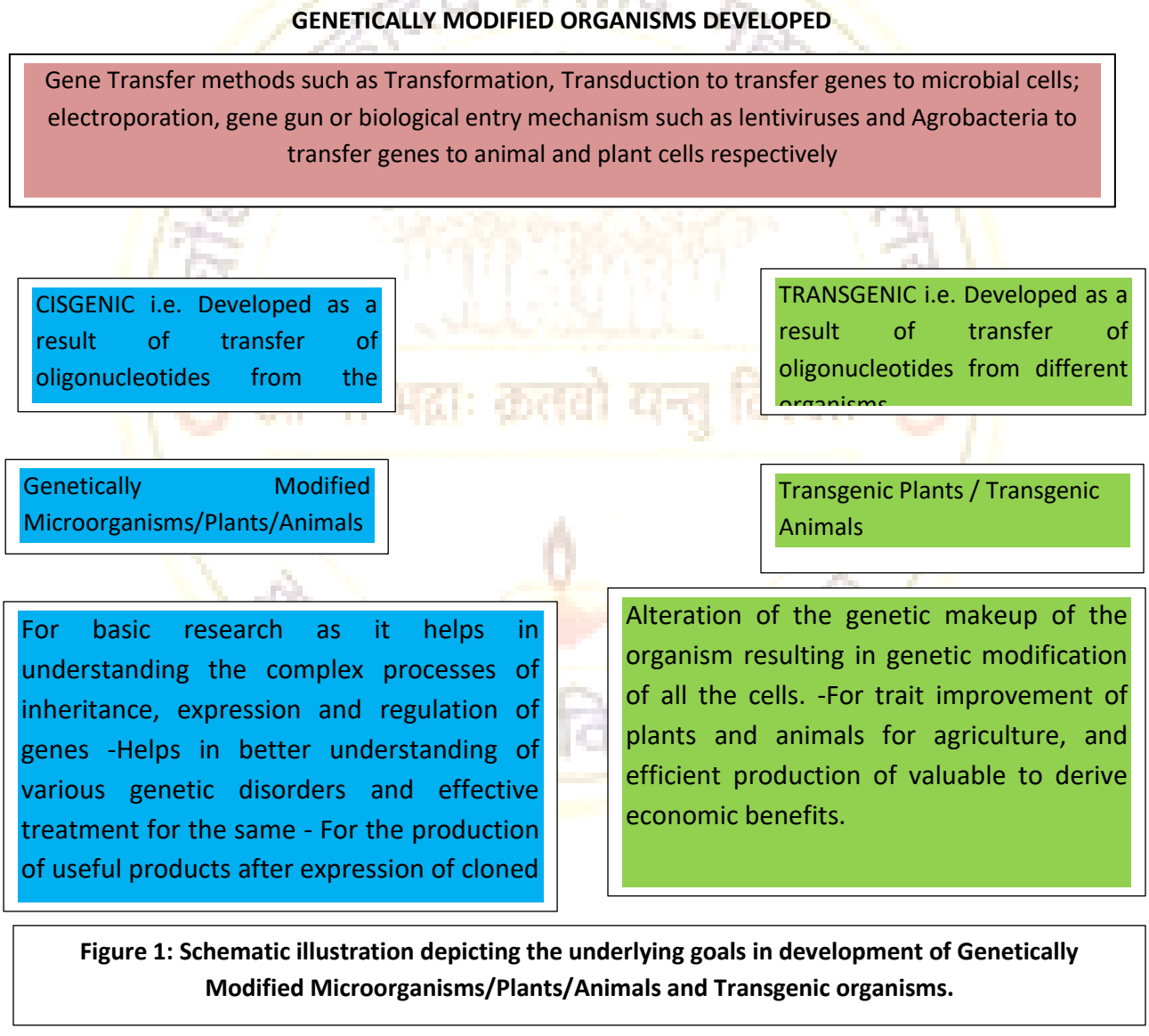


Figure 1: Schematic illustration depicting the underlying goals in development of Genetically Modified Microorganisms/Plants/Animals and Transgenic organisms.

GENERAL STRATEGY FOR GENE CLONING

To achieve the aims of genetic engineering, a cloning strategy has to be devised that will enable efficient use of the technology. There are basically four stages to any cloning experiment (Fig. 2.).

These are:
1. The isolation and preparation of DNA fragments
2. Production of recombinant by joining the fragments to a suitable vector
3. Introduction of the vector into a host cell
4. The screening of the recombinant host carrying the desired gene.

The successful completion of gene cloning results in a specific DNA sequence, which may be commercially applied for a variety of purposes like production of recombinant proteins, genetically modified microorganisms, transgenic plants and transgenic animals.

The most suitable bacterial host is Escherichia coli for the following reason:

- Complete knowledge of the gene structure,
- Faster growth rate,
- Easy to culture and handle

Besides the commendable characters of E. coli as a suitable host, the isolation and purification of the product becomes difficult owing to the presence of inclusion bodies in the host cell. Also, the expression of a eukaryotic gene becomes problematic in a prokaryotic host as many key elements of eukaryotic gene expression are generally absent in prokaryotes. These are:

- Chromatin and small RNAs regulation
- pre-mRNA processing
- Lack of intron region
- Lack of RNA-splicing machinery
- Post translation modification system

By cloning a cDNA form of the eukaryotic gene, the problem of intron can be avoided and incompatibility problems can be avoided by using eukaryotic cells as hosts. The use of eukaryotic host is the preferred choice and for this reason yeast, insect and mammalian cells are encouraged. Yeast cells are considered to be a suitable host as:

- Being single-celled fungi, they are easy to grow unlike most eukaryotes.
- The genome of yeast is designed to form Yeast artificial chromosomes (YACs) by combining the essential component of a eukaryotic chromosome like an origin site for replication, a centromere, and two telomeres which can be ligated with foreign DNA and thus serve as a vector.
- The YAC vectors can carry larger size eukaryotic foreign DNA compared to a plasmid.
- They are capable of doing post-translational modifications of the expressed eukaryotic proteins.

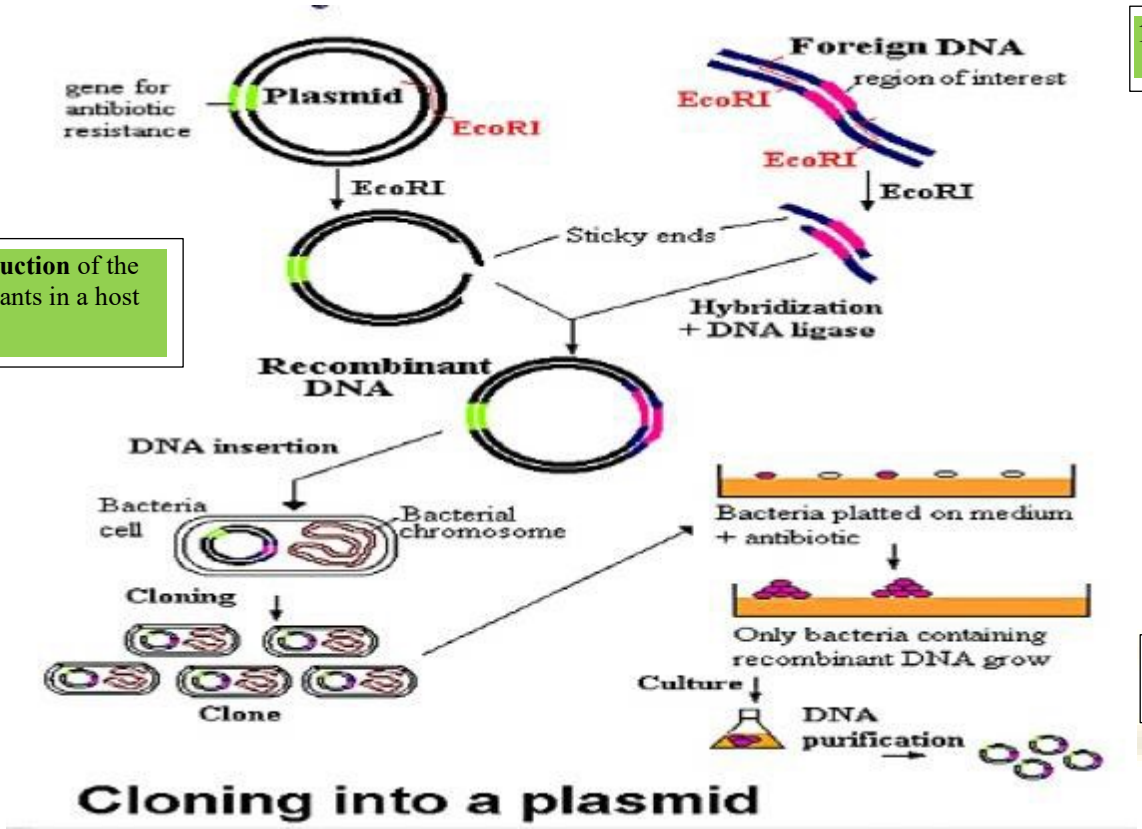


Figure 2: The different steps in a gene cloning.

However, eukaryotic proteins which require specific modifications, the successful cloning requires to be carried out in animal host. Dealing with animal and plant hosts the problem lies in the introduction of the foreign DNA into the organism. Hence, different techniques are employed to facilitate entry of foreign DNA into eukaryotic cells. Some of these techniques are:

- Direct DNA uptake involves the uptake of foreign DNA directly from the surroundings so the uptake is less efficient.
- Electroporation involves creation of a temporary hole in the plasma membrane by a brief electrical pulse through which foreign DNA can enter.
- Microinjection involves injection of DNA into individual cells using microscopically thin needles.
- Entry by biological means involves the use of infectious agents such as lentiviruses to transfer genes to animal cells or Agrobacteria to transfer genetic material to plants
- Once the DNA finds its entry inside the cell, it is incorporated into the cell's genomic DNA by genetic recombination.

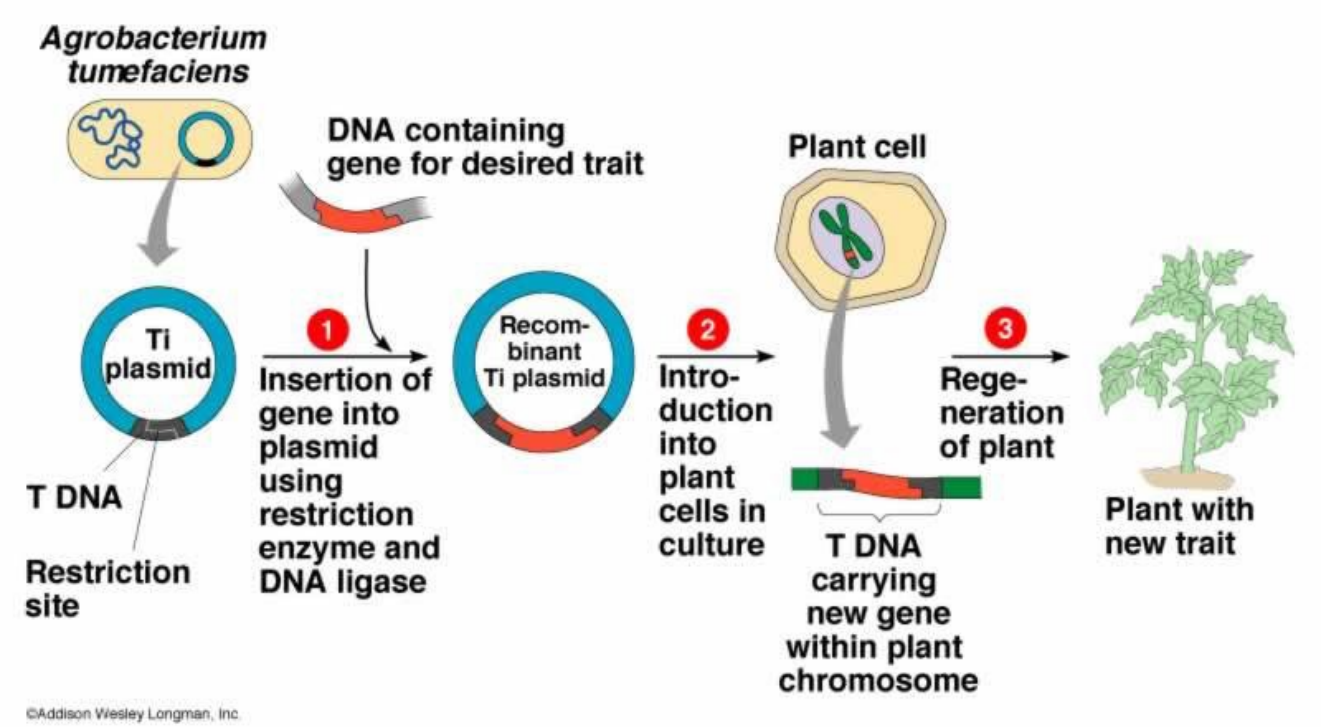


Figure 3: Cloning strategy in plants using Ti plasmid as a vector

History of GMO Development:

Paul Berg in 1972 produced the first recombinant DNA molecules. With advancement, it is now possible to manipulate, remove, and add genes to a variety of different organisms to induce a range of different traits. Herbert Boyer and Stanley Cohen in 1973 created the first genetically modified bacteria followed by a number of remarkable achievements in this field. Some of the major achievements of genetic engineering are:

- 1974: GM mice was created
- 1976: Commercialization of the technology after which producing and selling genetically modified foods and medicines began.
- 1982: The first commercial development of GMOs (insulin-producing bacteria)
- 1994: began to sell genetically modified food
- 1997:The first successfully cloned large mammal (sheep) named Dolly was developed
- 2003: began to sell GMOs as pets (Glofish)

A number of notable achievement since then involves development of drugs, such as treatments for cancer; development of transgenic insect-resistant crops; and development of transgenic animals for production of growth hormones and pharmaceutical products. The expression of trans-genes is possible in transgenic organisms as the genetic sequences for proteins are similar. The transgenic organisms are now developed to produce, various substances such as foods, pharmaceuticals, biochemical etc. Now it is possible to clone plants, fish, and even livestock.

Application of genetic engineering

The number of applications for genetic engineering are increasing as more and more is learned about the genomes of different organisms. A few interesting or notable application areas are shown in the fig.4.

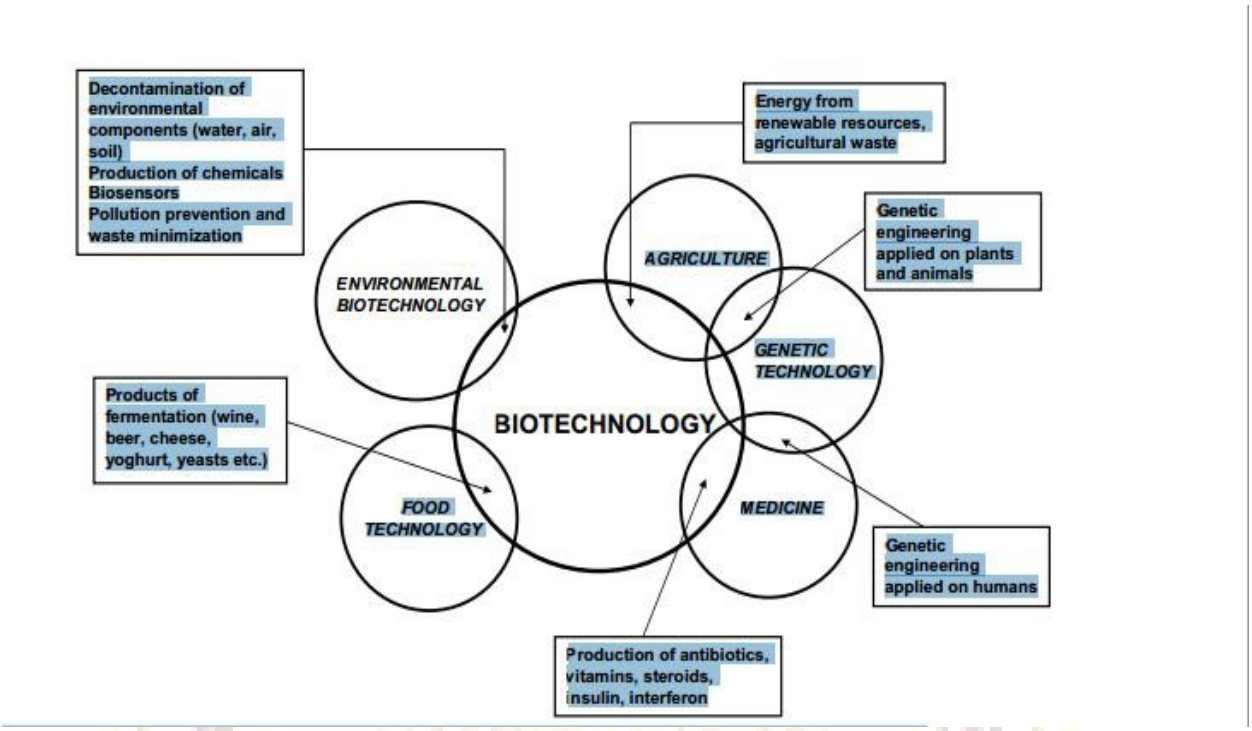


Fig.4. Application of Genetic Engineering in anthropogenic activities (**Industry, Agriculture, medicine, health, environment**).

Application in food industry

Genetic engineering finds application in food industry which is a result of modification of the genetic material of plants or animals. Many genetically modified (GM) whole foods or ingredients present in them available today are a result of gene modification.

A number of enzymes are involved in fermentation and digestion of foods. This has led to the concept of production of recombinant enzymes from genetically modified microbes such as chymosin and lipase for cheese production, and alpha- amylase for flavor enhancement in beer industry. A mixture of enzymes called Rennet is used to coagulate milk into cheese. This enzyme was initially available from the stomach of calves, and so was expensive, or from microbial sources, which caused unpleasant tastes. Genetic engineering has now made it possible to isolate and clone rennet-producing genes from animal into bacteria, fungi or yeasts to produce chymosin-a key enzyme present in rennet. A number of organisms like E. coli, Kluyveromyces lactis, and Aspergillus niger are cloned to produce recombinant chymosin. The first application of genetically modified organisms in food production was microbial enzymes which were approved in 1988 by the US Food and Drug administration. One of the latest technologies involves production of cow milk containing increased amount of a cheese making protein, casein and foods without beta-lactoglobulin (an allergen in milk) by RNA interference technology.

Genetically modified foods are obtained from genetically modified organisms, or transgenic crops. Genetic engineering has resulted in a number of improved traits in transgenic plants by genetic alteration. Some of these traits are:

- Production of extra nutrients in the food
- Increased growth rate

- Disease resistance and herbicide resistance
- Better taste
- Increased shelf life etc.
- Lesser requirement for water

The first genetically modified whole food crop was tomato (called Flavr Savr), which was made more rot-resistant. This was the first commercial genetically modified food marketed by Calgene as Flavr Savr delayed-ripening tomato in 1994. Genetic engineering mainly focusses on cash crops as shown in table. 1.

Table 1. List of Genetically Modified Foods

Genetically Modified Foods	Traits introduced
Rapeseed	Pesticides resistant; free from erucic acid
Cotton	Pesticides resistant
Sugar Cane	Pesticides resistant
Canola	Pesticides resistant; used in oil products, baked goods and snacks.
Flax	Herbicide resistant
Papaya	Resistant to virus
Tobacco	Presence of little nicotine
Meat	Production of meat from animals on modified diet
Peas	altered to produce a pesticide
Dairy Products	Production of growth hormone in cow milk.
Vitamins	Production of vitamins from foods like corn and soybeans.

Application in pharmaceutical industry and Medicine

By genetic engineering a variety of medical products are available today. Among these products, insulin and human growth hormone were first commercially available products obtained from recombinant E. coli.

Recombinant insulin is the result of successful genetic engineering. The initial production of insulin involved the separate synthesis of the insulin A- and B-chains in two bacterial strains. Both the insulin A and B chains genes were placed under the control of the lac promoter for inducible expression by lactose inducer. After purification of the A- and B-chains from the bacteria, the chains were then linked chemically to produce the final insulin. The production process of insulin is shown in Fig. 5. Recombinant-insulin is now commercially available in several forms and is involve in diabetes therapy.

The production of pharmaceutical products from transgenic animals is considered as "Pharming" (taken from "farming" and "pharmaceutical"). Pharming involves the use of genetic engineering technique to insert genes into host animals or plants resulting in expression of useful pharmaceuticals products. The development of transgenic "super mice" in 1982 led to the wide application of pharming in pharmaceutical industry. Human drug, TPA (tissue plasminogen activator) a valuable therapeutic protein to treat blood clots was first produced from "Super mice" as a result of genetic alteration in 1987. TPA is a protease that has a role in breaking down blood clots by breaking up fibrin, the protein involved in clot formation. Thus, TPA

is used in heart attack victims where it helps to reduce the damage caused by coronary thrombosis. Since then pharming has resulted in a plethora of recombinant products made in *E. coli* which is shown in table.2.

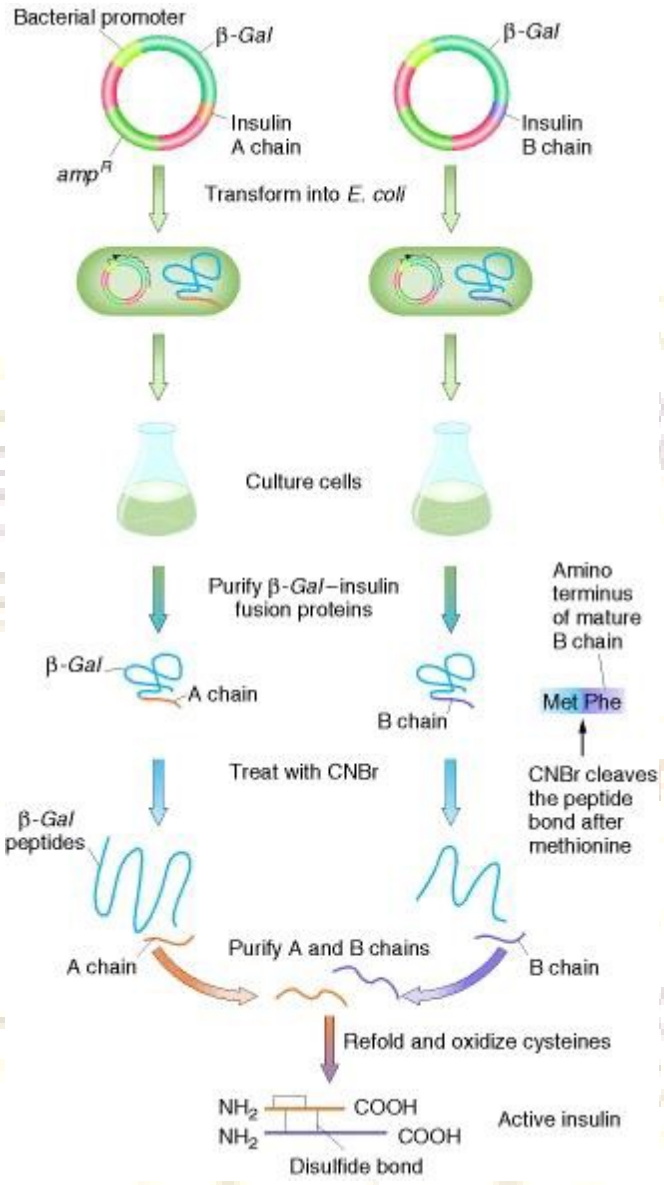


Table. 2. Recombinant products made in *E. coli*

Therapeutic Products	**Application**
Insulin	Treatment of diabetes
Haemoglobin	Used as a blood substitute
human protein C	Used as an anticoagulant

alpha-1 antitrypsin (AAT)	Treatment of AAT deficiency
Vaccines	Used as antigens
Growth hormones	Treatment of deficiencies
Factor VIII blood clotting factor	Used as blood clotting factor
Factor IX blood clotting factor	Used as blood clotting factor
Fibrinogen blood clotting factor	Used as blood clotting factor
Lactoferrin	Used as an infant formula additive
Tumor necrosis factor	Treatment of tumor cells
Interleukin-2 (IL-2)	Treatment of cancer, immune deficiency, and HIV infection
Taxol	Treatment for ovarian cancer
Interferon	Treatment for cancer; viral infections

The recombinant proteins produced by pharming acts as drugs for various human diseases. These therapeutic products can be directly injected into the bodies of the patient to treat the disease and deficiency.

The **recombinant vaccines** are an important group of therapeutic products. A number of vaccines are now available for animals, and human which is going to have a major impact in the healthcare industry. One of the initial vaccines produced by rDNA method involves the cloning of the surface antigen of the hepatitis B virus (HBsAg) in the yeast *S. cerevisiae* under the control of the alcohol dehydrogenase promoter. A number of recombinant vaccines are now commercially prepared by the recombinant DNA technology, where only the outside coat protein of the microorganism is expressed in the host to create the vaccine. The expressed protein can then be purified from the recombinant host and used for inoculation. This method has the advantage of safe delivery of antigen without transferring the actual disease-causing microbe to the host. Currently recombinant vaccines for the hepatitis B virus, herpes type 2 viruses, and malaria is under trial for use in future.

The latest development involves the production of **edible vaccine** using transgenic plants as a delivery mechanism, which involves the presence of vaccine in the edible part of the plant. This technology has tremendous potential as it enables easy delivery of vaccine by just consumption of the edible part. The trials for development of a vaccine-containing banana or tomato are currently under way.

With the advancement of genetic engineering it is now possible to treat the genetic defects by the replacement of the defective gene with a functional copy by **gene therapy**. This technique has great potential in the treatment of genetic diseases.

The gene therapy protocol can be made effective by the following approaches:

- Insertion of a normal gene to compensate for a nonfunctional gene
- Repair of an abnormal gene by selective reverse mutation

- Alteration in the regulation of gene pairs

It involves the following steps:

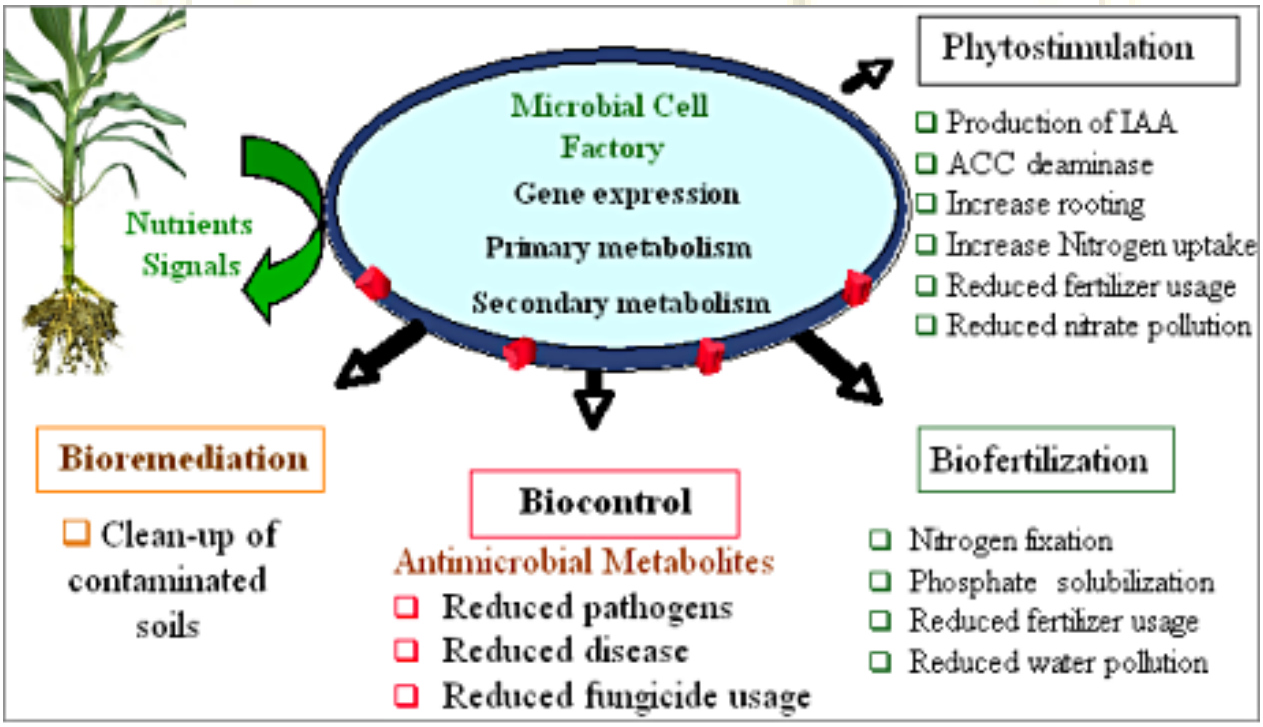
- Delivery of the therapeutic gene by a suitable vector into target cell of the patient
- Infection of the target cells with the viral vector
- Insertion of the genetic material into the target cell by recombination between the defective gene and the functional copy of the gene as shown in fig.6.
- Expression of functional proteins from the inserted gene resulting in a normal cell

A number of genetic disorder caused by single-gene defects, such as cystic fibrosis, muscular dystrophy, hemophilia, sickle cell anaemia and AIDS can be treated by gene therapy approach for which the clinical trial is underway. However, there are some problems associated to a successful gene therapy. These are: Introduction of large segment of DNA to the right site on the genome becomes problematic. Sometimes the defensive mechanism of the host destroys the genetically altered cells. Genetic engineering has thus resulted in different kinds of vaccines, antibodies and vitamins, drugs and hormones which are easily available in the market and are involved in the treatment of many diseases.

Application in Environment

Genetic engineering is exploiting the huge potential of microorganisms, plants, animals for the restoration of the environment. Genetic engineering is actively involved in the development of microorganisms and biocatalysts for remediation of contaminated environments, and in development of eco-friendly processes such as developing recombinant strain for bio-fuel production etc. Some of the areas where genetic engineering is involved are shown in Figure

Figure: Genetic engineering in Environmental restoration.



A number of genetically engineered microorganisms (GEMs) are developed which are involved in the bio degradation of waste materials. As the genes for enzymes involved in the bio-degradation pathway are mainly located on the plasmids, it is possible to create new strains by genetic manipulations of such plasmids. Using this technique, Chakrabarty and his team of co-workers in 1970s, developed a new strain of bacterium *Pseudomonas* named as "Superbug". This superbug is able to produce a combination of enzymes involved in degradation of a number of hydrocarbons present in petroleum. Superbug became the first genetically engineered microorganism to be patented.

Using the process of plasmid transfer it is possible to recombine two plasmids carrying gene for CAM (camphor degrading) and OCT (octane degrading) respectively. This results in a single CAM-OCT plasmid in the bacterium which can degrade both camphor as well as octane. The presence of heavy metals and other toxic organic materials present in the effluent is a major cause of concern for the aquatic life. Eutrophication is the result of accumulation of high levels of nitrogen and phosphorus in the effluents leading to undesirable growth of algae causing oxygen deficiency which is detrimental to the aquatic flora and fauna. Keeping this in mind, new recombinant strains of *Pseudomonas* have been developed which transforms a number of toxic chemicals such as hydrocarbons, chlorinated, solvents, polychlorobiphenyls and metals in a less toxic form. There has been a series of development of recombinant microorganism especially designed for the degradation of environmental pollutants. A summarized list of such microorganisms is shown in table.3.

TABLE.3. GENETICALLY ENGINEERED BACTERIA DESIGNED FOR THE DEGRADATION OF TOXIC WASTES

Bacterium	**Substrate**
P. putida	mono and dichloroaromatics
E. Coli	2,2,5-dichloropropionate
Alcaligenes sp.	Dichlorophenoxyacetic acid; 1,4- dichlorobenzene; mixed chlorophenols
Acinetobacter sp.	4-chlorobenzene
Pseudomonas capacia	2,4,5- trichoro-phenoxyacetic acid

Increased level of carbon dioxide is directly linked to global warming and greenhouse effect. So, efforts are being made to reduce the atmospheric CO2 concentration. In this context, the enzyme ribulose biphosphate carboxylase (RUBP-case) which is closely linked with CO2 fixation is being designed in a manner which results in increased photosynthetic efficiency. New strains of microalgae like mutants of *Anacystis nidulans and Oocystis sp* are being developed which can tolerate high concentrations of CO2.

Other modifications to bacteria include making changes to the cellular respiration process to alter the byproducts; typically, CO2 is produced, however engineers have made modifications so that hydrocarbon byproducts such as diesel and polyethylene (a fuel and a plastic) are produced.

Genetically modified organisms are used in clearing up of oil spills which is a major environmental hazard. New strains of *Pseudomonas* have been developed to break down a variety of hydrocarbons present at the oil-spill site, thereby decreasing the use of toxic chemical dispersants. Some microorganisms which are involved in the degradation of hydrocarbons are pseudomonads, corynebacteria and some yeasts.

The problem of soil pollution caused due to increased use of herbicides, pesticides and insecticides can also be solved by using recombinant microorganisms. The overuse of chemical herbicides, pesticides and fertilizers are detrimental to the environment so attempt is being made to develop **bacterial and viral pesticides** which will help in reduced use of chemical pesticides. Genetically engineered bacteria in which toxic genes from ***Bacillus thuringiensis*** is cloned are used as biological pesticides.

Application in Crop improvement through Transgenesis

Crop plants have been the focus of genetic engineering as efforts are being made to improve the traits of plants. Transgenic plants are developed for the following reasons:

- Gene insertion may result in improvement in the agricultural or commercial value of a plant.
- Transgenic plants can act as a living bioreactor facilitating production of commercially important proteins or metabolites.
- Transgenic plant helps in the understanding the function of different genes.

A number of genes can be combined with crops to produce desirable properties such as:

- Herbicide-, drought-, freeze- or disease-resistance
- Higher yield ; Tolerance towards cold, drought, salt
- Faster growth;
- Improved nutrition
- Delay of senescence
- Longer shelf life
- Increased post-harvest shelf life
- Altered flower pigmentation
- Nitrogen fixation Capacity to fix atmospheric nitrogen.

The creation of a transgenic plant involves:

- The Ti plasmid isolated from tumor-inducing (Ti) bacteria is used to transfer the gene.
- The modified plasmid is introduced into a cell.

The plasmid recombines with the plant genomic DNA. One remarkable development in transgenesis is the development of resistance against insect by introduction of gene responsible for the protein protoxin from Bacillus thuringiensis

Transgenic plants can be designed to produce a variety of useful compounds, like therapeutic products and metabolites. Recently transgenic crops with combined traits like herbicide tolerant and insect resistant has been developed.

Genetically Modified plant products in pipeline are:

- Increased levels of iron and vitamin A in rice.
- Fast ripening process in banana
- Improved feed value in maize
- High levels of flavonols in tomatoes
- Drought tolerance in maize

- Increased phosphorus availability in maize
- Plants more tolerant to arsenic
- Edible vaccines from plants
- Low lignin content in trees
- "Glowing plant" with gene from firefly that glows in the dark

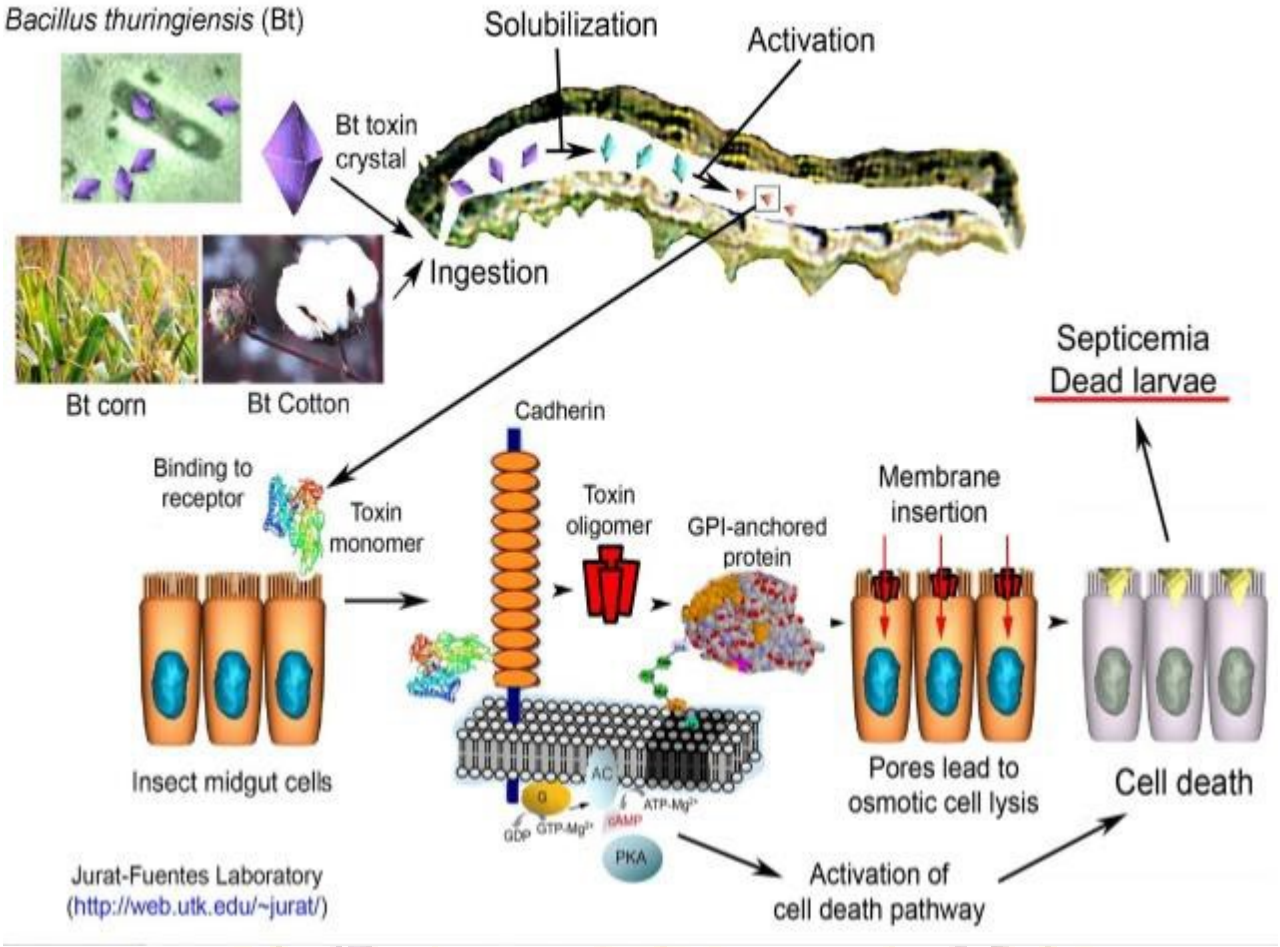


Figure: The development of insect resistance in plant by transfer of gene responsible for the protein protoxin from *Bacillus thuringiensis*.

Ethical consideration of genetic engineering

The characteristics of genetic engineering possess vast promise but also involve some potential threat to mankind bringing challenges to our ethical system and religious beliefs. The main reason genetically modified organisms are not more widely used is due to ethical concerns which are:

- GMO foods are not independently tested before the food is approved so the health of the humans is threatened because the consumption of it could cause allergic reaction.
- Another safety consideration is the health of farmers and their families, animals and communities who are put at risk with exposure to chemicals used in tandem with GMO seeds.

- The genetically engineered organisms which are released into the environment can be a threat to it because they can interact with other living organisms in the environment in an unpredictable manner.
- Genetic engineering can result in creation of toxic-vegetation, posing serious threat to wild life and may result in new strains of molds and fungi.
- Genetic engineering could be used to create biological weapons.
- The creation of pathogen resistance in plant may result in development of strong resistant variety of pathogens.
- There is serious concern that animals will suffer as a result of being genetically modified as use of growth hormones may cause limb deformation and arthritis as animals grow.
- Genetic engineering applied to human may have serious implications like parents opting for designer baby i.e. deciding their children's eye colors, heights or even genders before birth. Another concern is that if tests are carried out for genetic diseases on unborn babies – could lead to abortion if a disease is shown to be present.