Differences between Induction and Repression	
Induction	**Repression**
1. It is switching-on of an operon which is normally in switched-off state. 2. It starts transcription and translation. 3. It is caused by a new metabolite which needs enzymes to get metabolised. 4. It generally operates in a catabolic pathway.	1. It is switching-off an operon which is normally in switched-on state: 2. It stops transcription and translation. 3. It is caused by increased formation or availability of a metabolite. 4. It generally operates in an anabolic pathway.

The represser molecule has key role in regulation of lac-operon. Represser molecule active or inactive. Active represser may be rendered inactive by addition of an inducer while the inactive represser can be made active by addition of a co-repressor.Because the product of regulator gene the represser act by shutling off the transcripition of structural gene the operon model, as originally proposed by Jocob & Monad is referred as -negative control system.

GENE EXPRESSION IN EUKARYOTES

1. In eukaryotes, functionally related genes may not be clustered together constituting an operon.
2. The most popular model is known as **'Britten-Davidson model'** or 'Gene-battery model' proposed by Britten and Davidson in 1969.
3. A set of structural genes controlled by one sensor site is termed as battery.
4. Gene-battery model assumes the presence of four classes of sequences:
   (i) Producer gene: A producer gene is comparable to structural gene of prokaryotic operon.
   (ii) Receptor site: A receptor site is comparable to operator gene of bacterial operon and one such receptor site is assumed to be present adjacent to each producer gene.
   (iii) Integrator gene: Integrator gene is comparable to regulator gene and is responsible for synthesis of an activator RNA. It activates the receptor site.
   (iv) Sensor site: A sensor site regulates the activity of integrator gene. Activator gene can be transcribed only when the sensor site is activated.

The sensor sites are recognized by agents which change the patterns of gene expression like hormones and proteins. When a transcription factor (protein, hormone) bind to the sensor site it cause the transcription of integrato



Britten-Davidson model or Gene-battery model

GENETIC ENGINEERINGINTRODUCTION:

- Genetic engineering also referred as 'recombinant DNA technology' or 'gene splicing' is one kind of biotechnology involving manipulation of DNA.
- It deals with the isolation of useful genes from a variety of sources and the formation of new combinations of
  DNA (recombinant DNA) for repair, improvement, perfection and matching of a genotype.
- Thus, genetic engineering may be defined 'as a *technique for artificial and deliberately modifying DNA (gene) to suit human needs'*

- In genetic engineering manipulation breakage of DNA molecule at two desired places with the help of rectriction endonuclease to isolate a specific DNA segment and then insert it in another DNA molecule at a desired position.
- The new DNA molecule is recombinant DNA and the technique called genetic engineering. Genetic engineering aims at adding, removing or repairing of a part of genetic material. Genetic engineering can be used to improve the quality of human life.

Paul bergh (Father of genetic engineering). He transferred gene of SV 40 virus (simian virus) in to E.coli with the help of A, - phage. (Nobel prize - 1980)

The concept of genetic engineering was the outcome of two very significant discoveries made in bacterial research. These were-

(i) presence of extrachromosomal DNA fragments called **plasmids** in the bacterial cell,which replicate along with chromosomal DNA of the bacterium.

(ii) presence of enzymes **restriction endonucleases** which cut DNA at specific sites. These enzymes are, therefore, called '**molecular scissors'.**

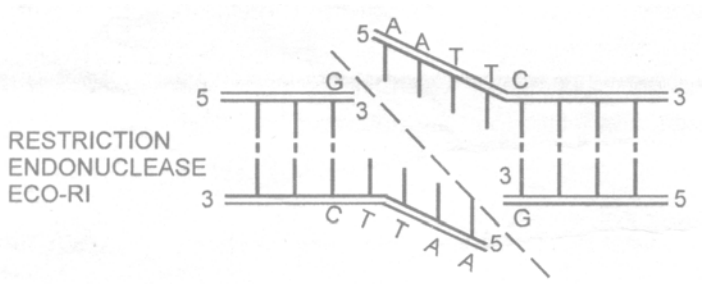
TOOLS AND TECHNIQUES OF GENETIC ENGINEERING

Genetic engineering involves cutting of desired segments of DNA and pasting of this D.N.A in a vector to produce a recombinant DNA (rDNA). The 'biological tools' used in the synthesis of recombinant DNA include enzymes, vehicle or vector DNA, passenger DNA and alkaline phosphatases.

1. Enzymes. A number of specific kinds of enzymes are employed in genetic engineering. These include lysing enzymes, cleaving enzymes, synthesising enzymes and joining enzymes.

(i) **Lysing enzymes.** These enzymes are used for opening the cells to get DNA for genetic experiment. Bacterial cell wall is commonly dissolved with the help of lysozyme

(ii) **Cleaving enzymes.** These enzymes are used for DNA molecules. Cleaving enzymes are of three types; exonucleases, endonucleases and restriction endonucleases.



(a) **Exonucleases** cut off nucleotides from 5' or 3' ends of DNA molecule.

(b) **Endonucleases** break DNA duplex at any point except the end.

(c) **Restriction endonucleases** cleave DNA duplex at specific points in such a way that they come to possess short single stranded free ends. For example, a restriction endonuclease ECOR-1 (from *Escherichia coli)* recognizes the base sequence GAATTC/CTTAAG in DNA duplex and cleaves it's strands between G and A.

Restriction enzymes are obtained from bacteria. They are useful to bacteria because the enzyme bring about fragmentation of viral DNA without affecting the bacterial genome. This is an adaptation against bacteriophages. g

Restriction enzyme (Eco R-I) was discovered by Arber, Smith & Nathans (1978 Nobel prize). These enzymes exist in many bacteria beside cleavage some restriction endonuclease, also have capability of modification. Modification in the form of methylation, by methylation the bacterial DNA modifies and therefore protects it's 5 own chromosomal DNA from cleavage by these restriction enzymes.

Restriction enzymes are used in recombinant DNA technology because they can be used in vitro to recognize and cleave within specefic DNA sequence typically consisting of 4 to 8 nucleotides. This specific 4 to 8 nucle-

Otide sequence is called **restriction site** and is usually **palindromic,** this means that the DNA sequence is the same when read in a 5'-3' direction on both DNA strand

AND MADAM DNA

As a result the DNA fregments produced by cleavage with these enzymes have short single stranded overhang at each end these kinds of ends are called sticky or cohesive ends because base pairing between them can stick the DNA molecule back together again.

```
      ↓
5GAATTC3'        5G3[1]                    5'AATTC3'
                ⇒ |                    +      |
3'CTTAAG5'       3 C T T A A 5'            3'G5'
      ↑
```

Therefore by cutting two different DNA samples with the same restriction enzyme and mixing the fragments together a recombinant DNA molecule can be generated.

Exceptionally, some enzymes cleave both strand of DNA at exactly the same nucleotide position, typically in the center of the recognition sequence resulting in blunt end or flush end.

Sma I (Serratia marcescens)

```
      ↓
5'CCCGGG3'      5'CCC3'      +      5' G G G 3'
               ⇒
3'GGGCCCS'      3'GGG5'      +      3'C C C 3'
      ↑
```

Nomenclature of enzyme - The first letter used for the enzyme is the first letter of the bacterium genus name (in Italics) then comes the first two letter of it's species (In Italics), next is the strain of the organism, last is Roman numerical signifing the order of discovery.

EXAMPLES OF RESTRICTION ENZYME

Recognition sequences of some restriction endonucleases

Name	Recognition sequence	End after cleavage		Source
EcoRI	-GAATTC- -CTTAAG-	-G -CTTAA -A	AATTC- G-	Escherichia coli - containing drug resistant plasmid RI.
Hind III	-AAGCTT- -TTCGAA-	-A -TTCGA	AGCTT- A-	Haemophilus influenzae
Bam I	-GGATCC- -CCTAGG-	-G -CCTAG	GATCC- G-	Bacillus amyloliquefaciens
Hae III	-GGCC- -CCGG-	-GG -CC	CC- GG-	Haemophilus aegyptius

(iii) **Synthesizing enzymes**. These enzymes are used to synthesize new strands of DNA, complementary to existing DNA or RNA template. They are of two types; reverse transcriptases and DNA polymerases.

(**a)** **Reverse transcriptases** help in the synthesis of complementary DNA strands on RNA templates:

(**b)** **DNA polymerases help** in the synthesis of complementary DNA strands on DNA templates.

(iv) **Joining enzymes.** These enzymes help in joining the DNA fragments. For example DNA ligase from Escherichia coli is used to join DNA fragments. Joining enzymes are, therefore, called molecular glues.

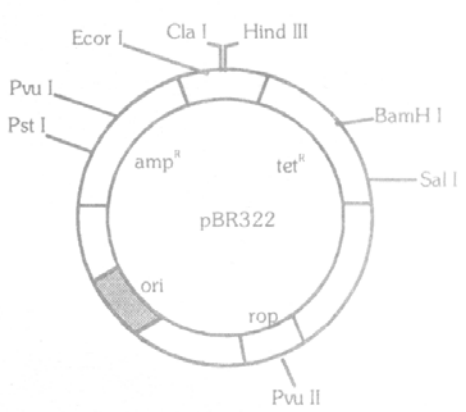
(iv) **Alkaline phosphatases**. These enzymes cut off phosphate group from the 5' end of linearised circular DNA and prevent its recircularisation.

2. **Vehicle DNA or Vector DNA.** The DNA used as a carrier for transferring a fragment of foreign DNA into suitable host is called **vehicle or vector DNA.**

You know that plasmids and bacteriophages have the ability to replicate within bacterial cells independent of the control of chromosomal DNA.

The following are the features that are required to facilitate cloning into a vector

(i) **Origin of replication (ori) :** This is a sequence from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within the host cells. This sequence is also responsible for controlling the copy number of the linked DNA. So, if one wants to recover many copies of the target DNA it should be cloned in a vector whose origin support high copy number.



(ii) **Selectable marker :** In addition to 'ori', the vector requires a selectable marker. Normally, the genes encoding resistance to antibiotics such as ampicilin, chloramphenicol, tetracycline or kanamycin, etc., are considered useful selectable markers for E. coli.

(iii) **cloning sites :** In order to link the alien DNA, the vector needs, recognition sites for the commonly used restriction enzymes. The ligation of alien DNA is carried out at a restriction site present in one of the two antibiotic resistance genes. For example, you can ligate a foreign DNA at the Bam H I site of tetracycline resistance gene in the vecter pBR322. The recombinant plasmids will loss tetracycline resistance due to insertion of foreign DNA (insertional inactivation) now, it can be selected out from non-recombinant ones by plating the transform ants on ampicillin containing medium. The transformants (plasmid transfer) growing on ampicillin containing medium are then transferred on a medium containing tetracycline. The recombinants will grow in ampicillin containing medium but not on that containing tetracycline. But, non-recombinants will grow on the medium containing both the antibiotics. In this case, one antibiotic resistance gene helps in selecting the transform ants.

Selection of recombinants due to inactivation of antibiotics is a cumbersome (troublesome) procedure because it requires simultaneous plating on two plated having different antibiotics. Therefore, alternative selectable markers have been developed which differentiate recombinants from non-recombinants on the basis of thtir ability to produce colour in the presence of a chromogenic substrate. In this, a recombinant DNA is inserted within the coding sequence of an enzyme, which is referred to as **insertional inactivation.** The presence of a chromogenic substrate X-gal (5-bromo-4chloro- β-D galacto pyranoside) gives blue Coloured colonies if the plasmid in the bacteria does not have an insert. Presence of insert results into insertional inactivation of the p-galactosidase (reporter enzyme) and the colonies do not produce any colour, these are identified as recombinant colonies.

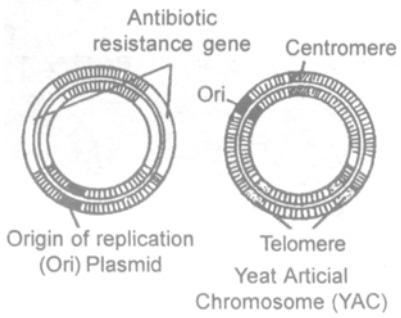
(iv) **Vectors for cloning genes in plants and animals :** Agrobacterium tumifaciens, a pathogen of several dicot

plants deliver a piece of DNA known as T-DNA' to transform normal plant cells into a **tumour** . Similarly, retroviruses in animals have the ability to transform normal cells into **cancerous** cells. A better understanding of the art of delivering genes by pathogens in their eucaryotic hosts has generated knowledge to transform these tools of pathogens into useful vectors for delivering genes of interest to humans. The tumour inducing (Ti) plasmid of Agrobacterium tumifaciens has now been modified into a cloning vector which is no more pathogenic to the plants but is still able to use the mechanisms to deliver genes (disarmed) and are now used to deliver desirable genes into animal cells. So, once a gene or a DNA fragment has been ligated into a suitable vector it is transferred into a bacterial, plant or animal host (where it multiplies).

(i) Plasmids. They are extra chromosomal DNA segments found in bacteria which can replicate independently. Plasmids can be taken out of bacteria and made to combine with desired DNA segments by means of restriction enzymes and DNA ligase. A plasmid carrying DNA of another organism integrated with it, is known as
recombinant plasmid or hybrid plasmid or Chimeric plasmid.

(ii) **Viruses.** The DNA of certain viruses is also suitable for use as a vehicle DNA. Bacteriophage (bacterial virus) has been used to transfer gene for P galactosidase from *Escherichia* co/i to human cells. Lambda
phage (A, phage) has been used for transferring lac genes of *E. coll* into haploid callus of tomato.

Vector type	**Insert size kb**
Plasmid	0.5 - 8
Bacterophage	9 - 23
Cosmid	30 - 45
BAC	50 - 300
YAC	1000 - 2500



3. **Passenger DNA.** It is the DNA which is transferred from one organism into another by combining it with the vehicle DNA. The passenger DNA can be complementary, synthetic or random.

(i) Complementary DNA (cDNA)- It is synthesized on *mRNA* template with the help of reverse transcriptase and necessary nucleotides. The DNA strand is then separated from the hybrid RNA-DNA complex by using alkali. Complementary DNA strand is then synthesized over the template of cDNA with the help of DNA polymerase. cDNA formed through reverse transcription is shorter than the actual or in ui'uo gene because of the absence of introns or non-coding regions.

(ii) **Synthetic DNA *(sDNA)-*** It is synthesized with the help of DNA polymerase on DNA template.
Kornberg (1961) synthesized first synthetic DNA from a mixture of deoxyribonucleotide triphosphates, DNA polymerase enzyme, metal ions and a segment of viral DNA.
Khorana (1968) synthesized first artificial gene (DNA) without a template. They synthesized the gene coding for yeast alanine *t-RNA*, which contained only 77 base pairs. However, it did not function in the living system. In 1979, **Khorana** was able to synthesize a functional tyrosine *t-RNA* gene of *E. coli* with 207 nucleotide pairs. Since then a number of genes have been synthesized artificially.

(iii) **Random DNA** - It refers to small fragments formed by breaking a chromosome with the help of restriction endonucleases.

Technique of Recombinant DNA

- The DNAs which are to be used as passenger DNA and the vehicle DNA are extracted out of their cells by lysing the cells with the suitable enzyme.
- The extracted DNAs are isolated from other cell contents by ultra centrifugation and purified.
- Both the passenger and vehicle DNAs are then, **cleaved by using the same restriction endonuclease so** that they have complementary sticky ends.

- The complementary sticky ends of the passenger and vehicle DNAs are joined with ligase enzyme. This gives rise to a recombinant DNA.
- The recombinant DNA is now inserted into a host cell such as Escherichia *coli*. The bacteria to be used as hosts should be without plasmids.
- The host cells are treated with calcium chloride or lysozyme. It creates transient (temporary) pores in their wall and makes the latter permeable to recombinant DNA.
- The recombinant DNA is added to the culture in which such bacteria are growing. The recombinant DNA is taken up by the bacteria. It replicates when the host bacteria divide and give rise to multiple copies of recombinant DNA.

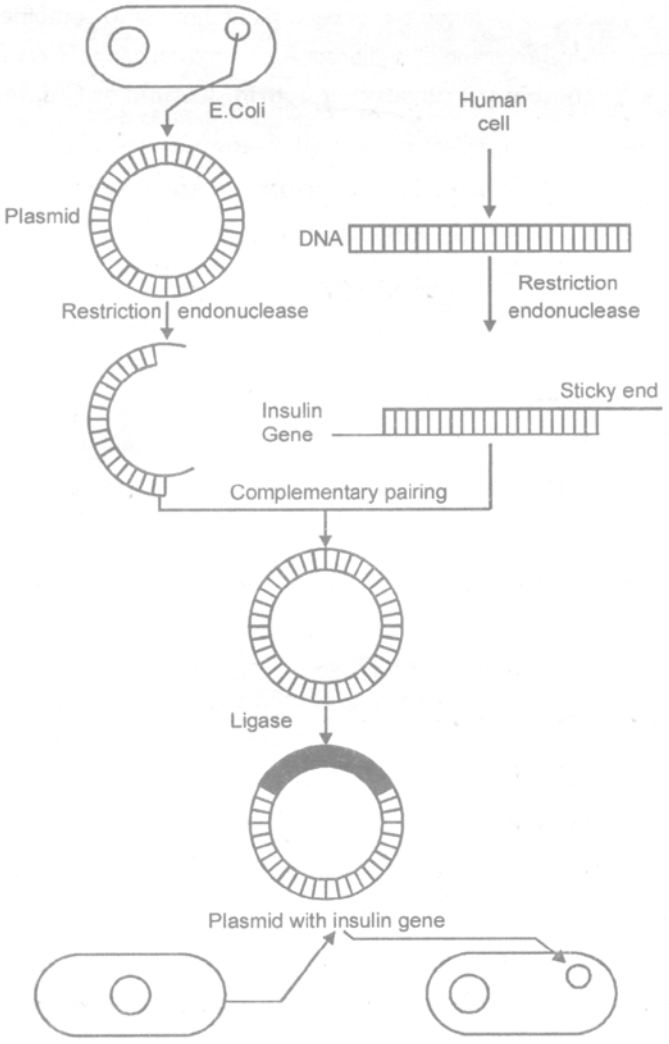


Fig.: Mechanism of transferring insulin gene from human DNA to E.coli with a plasmid

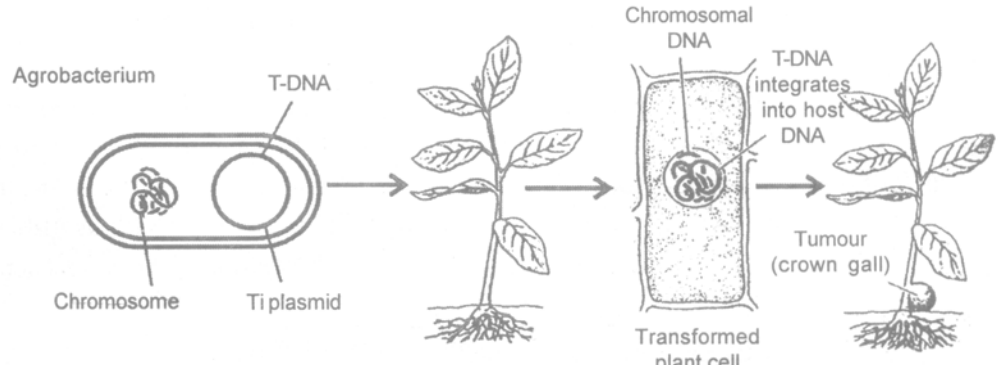
GENE TRANSFER

Transfer of desired genes from one organism into another is an important aspect of genetic engineering. Gene transfer involves essentially three stagas:

(i) Isolating a useful DNA segment from the donor organism.
(ii) Inserting it to a *suitable vector*.
(iii Splicing of this altered DNAs into a recipient organism or host cell.

The cell to begin making the appropriate product and then device an economical method for it's mass production. Gene transfer is achieved by two kinds of transfer methods: *(i)* **indirect** method throughvectors or carriers and *(ii)* direct or vector less transfer method.

(i) **Gene Transfer Through Vectors.** Plasmids and viruses are commonly used vectors for the transfer of desired genes. The desired genes are first made to join suitable plasmid or virus which are then introduced into the target cells.



- A plant pathogenic ***bacterium- Agrobacterium tumefaciens*** produces crown galls or plant tumours in almost all dicotyledonous plants.
- This bacterium infects all broad leaved agricultural crops such as tomato, soyabean, sunflower and cotton but not cereales.
- Tumour formation is induced by it's plasmid which is therefore called Ti plasmid (Ti for tumour inducing) *Agrobacterium tumefaciens naturally transfers some part of Ti-plasmid*

(a) T-DNA - (Transferred DNA)

(b) Vir-region - (Virulence region)

Inside the host plant cell T-DNA is seperated from Ti-plasmid, and integrated into host plant DNA that causes crown gall tumour.
Vir-region contains genes which are essential for T-DNA transfer and integration to host plant DNA. Vir-region contains 6-operons A, B, C, D, E, and G. (Acetosyringone is inducer of Vir-operon)
When we use Ti plasmid as a vector, first we remove the tumour causing gene from T-DNA region. Then desired gene inserted inplace of it. Now, this plasmid is called **disarmed plasmid.**
Same as Ri plasmid of **A.rhizogenes** (causing hairy root disease) also used as vector for gene transfer to plant cell.

(ii) Vectorless Gene Transfer

Foreign genes can also be transferred directly by the following methods:

(a) **Electroporation-** It creates transients (temporary pores) in the plasma membrane to facilitate entry of foreign DNA.

(b) **Chemical mediated genetic transformation-** It involves certain chemicals such as polyethylene glycol (PEG), tha help in the uptake of foreign DNA into host cells.

(c) **Microinjection-** It is the introduction of foreign genes into plant or animal cells using micropipettes or glass needles.

(d) **Particle gun-** It is a technique in which tungsten or gold particles coated with foreign DNA are bombarded into target cells to facilitate entry of the foreign genes.

(e) **Liposome mediatd gene transfer-** In this method DNA encloses within lipid begs. These lipid begs fused with protoplast.

GENE TRANSFER IN

In animals, the genes are transferred mostly through direct methods such as electroporation, microinjection or using particle gun.

The desired foreign genes can be introduced into fertilised eggs or embryos through microinjection. These transgenic eggs or embryos can be implanted into the uterus of another female, called **surrogate** (foster) mother for their further development. Now, since most of the human genes have been identified through **'human genome project',** it is hoped that a number of human genetic disorders such as Alzheimer, cancer, haemophilia, thalaessemia and cystic fibrosis can now be cured through insertion of the correct genes into these patients.

ACHIEVEMENTS OF GENETIC ENGINEERING

- The DNA recombinant technology or genetic engineering provides great benefits for advancement of science and society.

(1) A new system of medicine gene therapy, may develop to treat hereditary diseases such as haemophilia. Genetic disorder can be over come by introducing specific gene.

(2) Bacteria may be used as **"Living factories"** for synthesizing vitamins, hormones and antibodies.

- Human insulin **(Humulin)** was first genetically engineered product produced by an American firm **Eli Lilly** - 5th July 1983.
- **Charles weismann** of university of Zurich, obtained **interferon** through recombinant E.coli (1980)
- Microbes have been engineered to produce Human growth Hormone **(HGH)** for curing dwarfism.
- **Vaccines** which are produced by genetic engineering e.g., for Hepatitis-B and Herpes virus).

Nitrogen fixation genes may be transferred from bacteria to the major food crops to boost food production without using expensive fertilizers.

- Transgenic plant obtained through recombinant DNA technology. **First transgenic plant was tobacco.** It contains resistant gene against weedicide (Glycophosate). First transgenic animal was mouse contain gene for growth hormone. First introduced transgenic crop in India (2002) is **Bt-cotton.**

It is resistant for boll worm(Helicoperpa armigera - Larva of insect). It is formed by transfer of pest resistant gene from Bacillus thuringiensis (bt-2 gene encoding Bt-toxin).

Bacillus thuringiensis produces a toxic protein called crystal protein (Cry-Protein) this protein is toxic for larva of certain insect.

This protein kills the insect by inhibiting ion transport in midgut (bt 2 gene is called cry-gene)

- In pollution control, microbes have been engineered to break up the crude oil spills.

Dr. Ananda Mohan chakraborthi introduced plasmid from different strains in to single cell of **pseudomonas putida.** The result was new genetically engineered bacterium which would cleaning oil spills called **"Super bug"** (oil eating bug.)

Applications of Recombinant DNA products

Medically useful recombinant products	Applications
Human insulin	Treatment of insulin - dependant diabetes
Human growth hormone	Replacement of missing hormone in short stature people.
Calcitonin	Treatment of rickets.
Chorionic gonadotropin	Treatment of infertility.
Blood clotting factor VIII/IX	Replacement of clotting factor missing in patients with Haemophilia A/B.
Tissue Plasminogen activator (TPA)	Dissolving of blood clots after heart attacks and strokes.
Erythropoetin	Stimulation of the formation of erythrocytes (RBCs) for patients suffering from anaemia during dialysis or side effects of AIDS patients treated by drugs.
Platelet derived growth factor	Stimulation of wound healing
Inter feron	Treatment of pathogenic viral infections, cancer
Interleukin	Enhancement of action of immune system
Vaccines	Prevention of infectious diseases such as hepatitis B, herpes, influenza, pertusis, meningitis, etc.

Application of Genetically Engineered Microbes

Microbes	Applications
Escherichia coli (gut bacterium)	Production of human insulin, human growth factor interferons, interleukin and so on.
Bacillus thuringiensis (soil bacterium)	Productions of endotoxin (Bt toxin), highly potent, safe and biodegradable insecticide for plant protection.
Rhizobium meliloti (bacterium)	Nitrogen fixation by incorporating "nif" gene in cereal crops.
Pseudomonas putida (bacterium)	Scavenging of oil spills by digesting hydrocarbons of crude oil.
Bacterial strains capable of accumulating heavy metal	Bioremediation (cleaning of pollutants in the enviroment).
Trichoderma (fungus)	Production of *enzyme* chitinases for biocontrol of fungal diseases in plants.

Transgenics and their potential applications

Transgenic	Useful appications
Bt Cotton	Pest resistance, herbicide tolerance, and high yield.
Flaur Savr Tomato	Increased shelf-life (delayed ripening) and better nutrient quality
Golden Rice	Vitamin A and Fe - rich
Catties (cow, sheep, goat)	Therapeutic human proteins in their milk
Pig .	Organ transplantation without risk of rejection

Medical Diagnosis of Disease