Handwritten Notes On Application of Biotechnology

APPLICATIONS OF BIOTECHNOLOGY

[r-DNA Technique / Gene transfer tech.]

Applications
↳ have been observed in many diff. fields.
→ agriculture / Medical science.
(Food science) (Biopharmaceutical)
→ Environmental science / Bioremediation
(pop pollution control)

Applications in:

Green Biotech :: agriculture
Red Biotech :: Medical science
White / Grey Biotech :: Industry
Blue Biotech :: Marine science

[I] application of Biotech. in agriculture:
(3) types.
① agrochemical based agri.
② organic agriculture
③ genetically engineered crops based agriculture. (GMO)
↓
GMF (food)

GM crops are produced:
① To enhance nutritional quality of food.
eg. Golden Rice
↳ Rich in vit. A
↳ carotene gene was transfered.

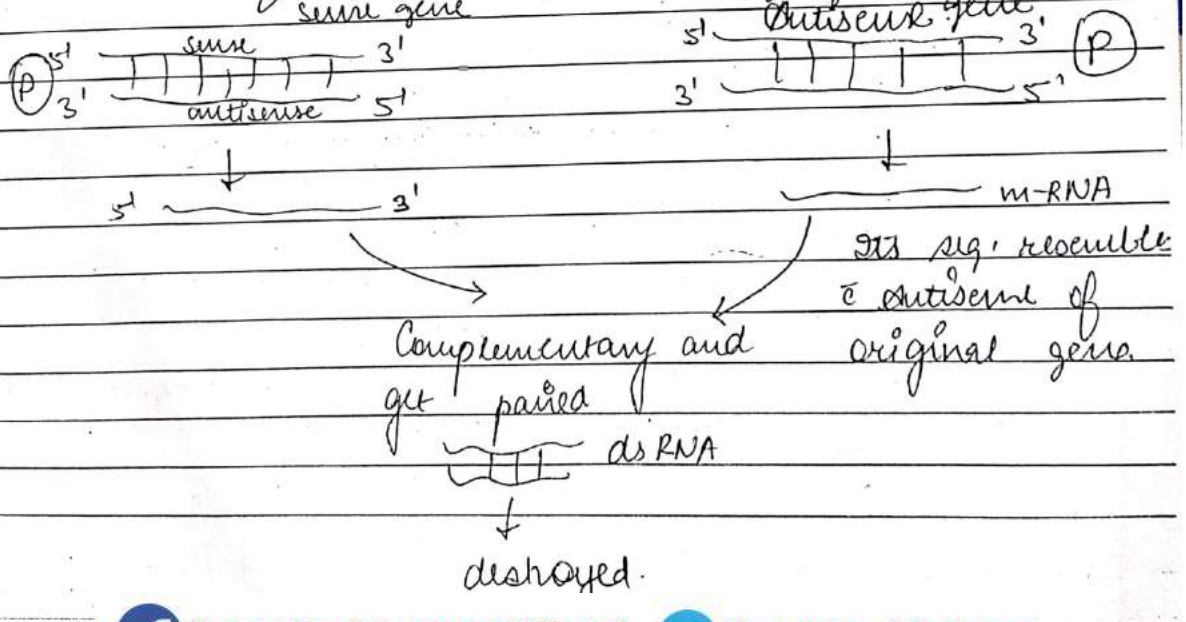
Protein Rich Potato
↳ overcome

(2) To develop Tolerance against abiotic stresses (flood, drought, frost, salinity etc.)

eg: Salt Tolerant Transgenic Tomato
↳ In this a gene was transferred which highly expressed Na^+-K^+ antiport pump in its membrane by which it accumulates all additional ions in its vacuole and later on release them out.

(3) To reduce post Harvest losses
eg. Flavr - Savr Tomato
↳ have long self life due to delayed ripening

Antisense Technique
In this the expression of gene of ripening eg; polygalactouronase slow down by the use of antisense technology



(4) To develop Herbicide Resistant plant.

eg. Herbicide Resistant Tobacco (1st Transgenic plant)

↓

Sulphonyl urea and Glycophosate } plant herbicides

In this ALS gene of Arabidopsis was transferred.

ALS → Acetolactate synthetase

(5) For production of Biopharmaceutical compounds:

eg. gene of Hirudine was transferred in Brassica Napus (Rapeseed oil)

↓

It produces Hirudine which collects in its seed.

↓

Seeds are crushed.

↓

Oil obtained.

↓

Hirudin seperated.

↳ used as Blood anticoagulant.

eg. Edible Vaccine → Trans. Banana] for Hepatitis and cholera

→ —"— Tomato]

→ —"— Sugarbeet → for foot & mouth dis.

→ —"— Banana] for Diarrhoea

← —"— potato]

⑥ To develop Pest Resistant plants
→ Insect Resistant plants (Bt plants / crops)
→ Nematode —"— —"—

1st Biopesticide developed by Bt.

In them a Bt gene / Cry protein of Bacillus thuringiensis Bact. is transferred.

↓

It produces a toxic, insecticidal protein which kills many insects

Insects of three groups →
① Lepidopterans (army worm, tobacco bud
② coleopterans (Beetles)
③ Dipterans (flies, mosquito

This protein never kills its own Bacteria because in Bacteria it is found in insoluble inactive crystal forms (endotoxin) (Protoxin) ∴ this protein is known as crystal protein / Cry protein

Insect Resistant plants
When insect feeds this protein the insoluble protein gets dissolved in its midgut due to alkaline med. and it becomes active, this active pt. binds to midgut epi. creates pores causes swelling and lysis of gut and thus ultimately insect will die.

↳ Cry gene → Insect group specific
→ several types of Cry genes.
→ selection of gene depends on:
Targeted Insect Host plant

eg: Cry IAc gene and Cry IIAb gene → for Bollworm
Cry IAb gene → for corn Borer insect.

* a suitable cry gene is sucked and isolated from Bacteria and transferred in host by Ti-plasmid vector.

eg:
- Bt cotton
- Bt tobacco
- Bt soyabean
- Bt corn
- Bt rice
- Bt Brinjal
- Bt Mustard

} these are all resistant to insects.

Bt cotton ⇒ Resistant to Bollworm.

Nematode Resistant plants

↳ Cry also causes diseases in some plants. (only few are pathogenic / pest)

eg: Meloidegyne incognitia
↳ causes Red Knot disease in Tobacco.

Nematode resistant plants are developed by its a way special technique RNA Interference (RNAi) Technique.

↓

It is a natural method of cellular defence in Eukaryotes.

↓

studied in detail by :
Fire and Mello
(2006 → Nobel prize

RNAi → It is the technique of gene silencing

↓

In this the expression of any gene is inhibited. by destroying its m-RNA.

↓

This m-RNA is destroyed by a complementary ds RNA

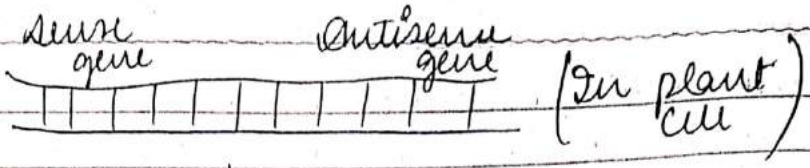
Source of ds complementary RNA.

↙ Artificially and transferred in cell with the help of some vector like Retrovirus / Transposons.

This ds RNA taken on initiates RNAi in cell & causes gene silencing.

↘ as a sense and antisense gene is introduced in a cell which forms a ds RNA inside the cell and this ds-RNA initiates RNA i in cell.

vector used: transposons, retrovirus.



↓

m-RNA

↓

ds RNA

↓

To this, Dicer protein binds which cleave it into fragments

↓

SiRNA
(Short Interference RNA)

↓

To this, a multicomponent nuclease enzyme compound binds (More Helicase activity) It removes one one strand of dsRNA.

RISC (RNA induced Silencing Compd.)

In plants

(nematode gene)
Targeted gene

↓

m-RNA

Bind to m-RNA

RISC

m-RNA

It cuts mRNA into fragments

↓

gene get silenced ← m-RNA degraded

(Red Biotech.)

Application of Biotechnology in Medical Science.

→ Involves formation of medicines.
→ Treatment of disease.
→ Diagnosis of disease
→ Formation of medicines.
↳ Recombinant Therapeutics
↳ Very effective
↳ pure
↳ safe
↳ Low side effects

So far about 30 recombinant medicines are being used all over the world.
In India only 12 recombinant medicines are used.

eg. genetically engineered Insulin :
↓
proteinaceous hormone which regulate blood glucose level.

If insulin is less in amount then glucose level increases in Diabetes ~~Do~~ Mellitus. Then it is given from outside
↓
Initially it was obtained from cattles and pigs (Bovine insulin)
↓
was usually allergic to human beings.

natural method of Insulin formation in Humans.

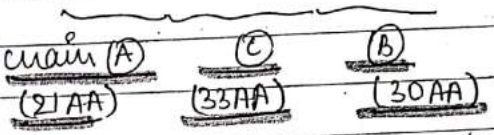
↳ Insulin.

↓

Its gene is present on short arm of chromosome 11.

↓

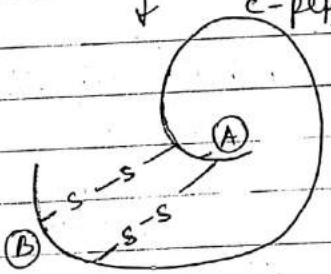
↓ Translation



PRODNSULIN (Inactive)

↓ c-peptide

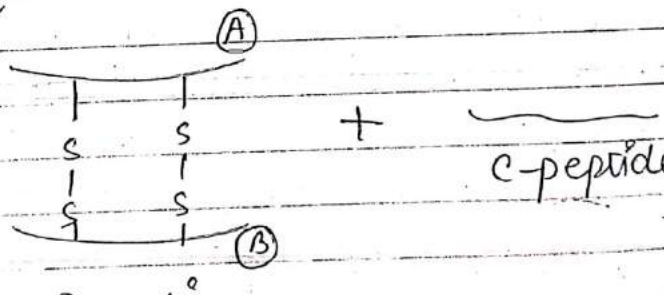
Formation of Disulphide Bond



only in Humans not in Bacterias.

(Removal of C-peptide (By β-cell peptidases))

↓



Insulin

↳ digested in stomach.

(7)

Genetically Engineered Insulin
↳ developed by an american pharma company Eli Lilly (1983)

They developed two artificial gene of chain A and chain B and transferred them into two separate E.coli.

Artificial DNA/gene of chain (A) → plasmid vector → in E.coli → chain (A) obtained

chain (B) → plasmid → in E.coli → chain (B) obtained.

Joined by Disulphide bond (most challenging step)

↓

Insulin formed 5th July 1983. HUMULIN (1)

(2) HUMALOG - more purified than Humulin (By - Eli Lily & Ranbaxy)

② Gene Therapy → method of Treatment of some genetical dis.

→ It is the collection of all those methods which are involved in treatment of a genetic disorder that has been identified in an embryo/child

★ → 1st successful gene therapy was done in 1990 in a 4-yr old girl suffering from ADA deficiency.

↓

- Lack of enz. adenosine deaminase

↓

Used Immunity - req. for the formation of functional T-lymphocytes from Bone marrow cells.

results in "SCID"

"Severe Combined Immuno deficiency"

Treatment:

① Enz. Replacement therapy
② Bone Marrow transplantation
③ Gene Therapy

Affected person → Bone Marrow cells are taken → ADA gene is transferred by Retrovirus vector → Selection of Recom Bone Marrow cell → Culture (some cells are maintained in culture.) ← Reinforced in person again.

For the permanent cure this gene transfer should be done at Embryonic stage.

(3) Molecular Diagnosis

Here the techniques of Biotechnology are used for very early and accurate diagnosis of any disease / pathogen in any human.

Technique → { r-DNA, PCR, ELISA }

- In disease diagonosis in Adult (After birth)
  - DNA pattern on X-Ray film → +ve test. (Disease confirmed)
- In disease diagnosis in Embryonic Stage (Before Birth)
  - DNA pattern on X-Ray film → -ve test. (Disease X).

Small, singe stranded radioactive DNA / RNA probe are used.

TRANSGENIC ANIMALS.

Less in comparison to plants and microbes.

Mainly, Mice → more than 95%
Sheep
goat, cow, pigs etc.

*Uses and applications

① For the production of some important Biological / Industrial product.

Organism	gene Transferred (Human)	Uses
Trans. sheep	Blood clotting factor VIII/IX gene	Treatment of Haemophilia
Trans. sheep	Human α-1 antitrypsin gene	Treatment of Emphysema
Trans. cow	Human Lactoferrin gene	Treatment of cystic fibrosis
Trans. cow (Rosie) → produces Nutritious milk rich in lactalbumin (2.4g/L)	Human Lactalbumin gene	used in infant feeding.
Trans. goat	TPA gene (Tissue plasminogen activator gene) ⇓ Streptokinase	used for the removal / dissolution of blood clot in heart / BV.
Trans. goat	Spidar gene	Thread formed (used for clothes & other Products)
Trans. pig	Human Antigen gene	Their organs are used for transplantation.

Trans. sheep / pig / etc. → Human growth → size ↑↑
cow / fishes hormone used as food.
↳ Trans foods are known as GMF → food.

(2) For the study of normal physiology and development.
↳ Study of gene expression and regulation
Transgenic mice are studied.

(3) ↳ For the study of diseases.

eg. Oncomouse → for cancer study

(4) For vaccine safety testing
eg. Transgenic mice had been used for safety testing of polio vaccine.

(5) For chemical safety / Toxicity Testing

In this sensitive transgenic organisms are developed by gene transfer and then they are used for safety testing.

TRANSGENIC MICROBES.
↳ Max. in no.

E. coli
~~Trans~~ Transgenic
① having Human Insulin gene → Treatment of Diabetes
② having human growth hormone → Treatment for Dwarfism

Pg 18 - Module

Transgenic E-coli
- ③ having calcitonin gene → Treatment of Rickets
- ④ having interferon gene → For viral Resistance
- ⑤ having antigen gene → For Vaccine production

★A Pseudomonas putida (Superbug) → 1st Biopatent
- → Developed by → Anand Mohan Chakravarty
- ↳ It can digest hydrocarbon / petroleum
- ↳ It is used to remove oil spills from oceans. Marine water pollution control.
- ↳ an eg. of Bioremedial (use of living org. to remove pollution) @ Bioremediation.

→ In this bacteria ④ types of genes / Plasmids were transferred.
1. OCT gene → Octane digestion
2. XYL gene → Xylene digestion
3. CAM gene → camphor digestion
4. NAH gene → naphthalene digestion

GEAC

Basmati Rice