

Biotechnology & its Applications

Biotechnology = industrial scale production of biopharmaceuticals & biologicals using GM microbes, fungi, plants & animals ~~only~~.

Three critical research areas

- (i) best catalyst → improved organism or a pure enzyme.
- (ii) optimal conditions for catalyst to act.
- (iii) downstream processing to purify the protein / organic compound.

Applications in Agriculture

Three options to increase food production :

- (a) agro-chemical based
- (b) organic farming
- (c) genetically engineered crop-based.

Green Revolution → 3x food supply

← still not enough
← for growing pop.

Yield rose due to : better varieties, management practices & agrochemicals (fertilisers + ~~herbicides~~ pesticides) .

BUT agrochemicals → costly + harm soil, water, non-target species. GM is alternative.

GM Crops (GMOs)

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Plants / bacteria / fungi / animals whose genes have been altered by manipulation = Genetically Modified Organisms (~~GMAA~~ GMO) .

GM has helped to :

- (i) tolerate abiotic stress (cold, drought, salt, heat).
- (ii) reduce dependence on chem pesticides (pest-resistant crops).
- (iii) reduce post-harvest losses.
- (iv) raise mineral-use efficiency of plants.
- (v) ^{*} enhance nutrition e.g. golden rice (Vitamin A enriched rice).

Bt Cotton

Bacillus thuringiensis → makes Bt toxin which kills lepidopterans (bollworm, armyworm), coleopterans, dipterans.

cry I Ac, cry II Ab → ~~boltwstnt~~ Ab →
← corn borer

Toxin is made as inactive prototoxin (crystal).

In insect midgut → alkaline pH solubilises it

→ active toxin → pores in gut cells → death.

Pest-Resistant Plants : RNAi

Nematode *Meloidogyne incognitia* infects tobacco roots \rightarrow heavy yield loss.

RNA interference (RNAi)

RNAi = cellular defence in all eukaryotes :

silencing of a specific mRNA by a

complementary dsRNA molecule that binds it & prevents translation.

Source of dsRNA : viruses with RNA genomes or mobile elements (transposons).

sense RNA + anti-sense RNA \rightarrow dsRNA \rightarrow RNAi

Strategy used

Using *Agrobacterium* vectors, nematode-specific genes were inserted in host plant.

DNA was made so as to produce ~~only~~ both sense & anti-sense RNA \rightarrow form dsRNA \rightarrow silence specific mRNA of nematode.

Parasite cannot survive in the transgenic host \rightarrow plant is protected.

gene \longrightarrow dsRNA \longrightarrow RNAi \longrightarrow no parasite

Genetically Engineered Insulin

Insulin manages adult-onset diabetes.

Earlier : extracted from pancreas of slaughtered ~~sheep~~ cattle & pigs.

Problem : caused allergy / immune reaction.

Structure of insulin

Two short polypeptide chains :

chain A + chain B joined by
DISULPHIDE bridges (-S - S -).

pro-insulin → -C peptide →

insulin^{peptide} =
← extra bit

In mammals → insulin made as PRO-hormone

(pro-insulin) with extra C peptide. Mature

insulin has C peptide removed. This was the challenge with rDNA insulin production.

Eli Lilly (1983) → Humulin

Prepared two DNA sequences = A chain

+ B chain of human insulin. Both inserted

into plasmids of E. coli → chains produced

separately → extracted → joined by

DISULPHIDE bonds ⇒ mature human insulin.

Note : insulin must be injected (peptide).

Gene Therapy & Diagnosis

Gene therapy

Correction of a gene defect in a child or embryo by inserting genes into cells/tissues.

1st clinical gene therapy - 1990 - a 4-yr old girl with ADA deficiency.

ADA = adenosine deaminase ; vital for immune system. Disorder = deletion of ADA gene.

lymphocytes + ADA cDNA <- via retro-
<- viral vector

Lymphocytes are not immortal -> periodic infusion needed. Permanent cure : isolate gene from marrow cells (embryonic stage).

Molecular diagnosis

Conventional tests detect a disease late.

Recombinant tools - early & accurate.

(a) PCR - amplify ^{*} pathogen nucleic acid in suspect ; detects ^{*} HIV / cancer / mutations even at very low concentration.

(b) ELISA - antigen-antibody interaction ; detects infection by antigen / antibody.

Transgenic Animals

Animals whose DNA has been manipulated to carry an ~~extra~~ foreign gene = transgenic.

95% are* mice ; also rats, rabbits, pigs, cows, sheep and fish.

Why are they made ?

- (i) study normal physiology / gene regulation & how genes affect body.
- (ii) study disease - *models for cancer, cystic fibrosis, rheumatoid arthritis, Alzheimer's.
- (iii) biological products - e.g. human alpha-1 antitrypsin (emphysema), factor VIII (haemophilia), insulin in milk.

Rosie (1997) - cow, 2.4 g/L

<- human alpha-
<- lactalbumin

Rosie's milk was more nutritionally balanced for human babies than natural cow's milk.

(iv) Vaccine safety testing

Transgenic mice are being used to test polio vaccine safely -> may replace monkeys.

(v) chemical safety testing - toxicity tests.

Ethical Issues & GEAC

Modifying living organisms has unpredictable outcomes when introduced into ecosystems.

Ethics = moral standards for evaluating whether such ~~acts~~ actions are right.

GEAC

Genetic Engineering Approval Committee -
Indian govt. body that decides validity of GM research & safety of releasing GM organisms for public use.

Biopiracy & Patents

Biopiracy : MNCs use bio-

resources w/o permit / payment

Developing nations = rich in bio-resources & traditional knowledge. Developed nations are poor in these but rich in tech & funds.

Examples : Basmati rice - US patent (RiceTec) based on Indian varieties ; turmeric, neem patents - all challenged by India.