CHAPTER-12

BIOTECHNOLOGY AND ITS APPLICATIONS

Biotechnology has varied applications, some of which include-

i) Therapeutics ii) diagnostics iii) genetically modified crops for agriculture iv) processed food v) bioremediation vi) waste treatment vii) energy production

There are three critical research areas of biotechnology:

i) Providing best catalyst as improved organism, usually a microbe or pur enzyme.
ii) Creating optimal conditions by engineering for a catalyst to act.
iii) Downstream processing technologies to purify the protein/organic compound.

1. Biotechnological applications in agriculture

Food production can be increased by applying biotechnology by the following ways

a) Agrochemical-based agriculture
b) Organic agriculture
c) Genetically engineered crop-based agriculture.

The green revolution succeeded in increasing food supply because of-a) use of improved crop varieties. b) use of agrochemicals (fertilisers and pesticides). c) use of better management practices.

Agrochemicals are expensive for farmers in developing countries and also have harmful effects on environment. Therefore, genetically modified (GM) crops were developed. Genetically modified organisms (GMOs) are plants, bacteria, fungi and animals whose genes have been altered by gene manipulation.

Genetic modification of crops have resulted in: a) increased tolerance against abiotic stress (cold, draught, salt, heat) b) reduced reliance on chemical pesticides (pest-resistant crops) c) reduced post-harvest loses d) increased efficiency of minerals used by plants (preventing early exhaustion of fertility of soil) e) enhanced nutritional food value, e.g., vitam A enriched

rice (golden rice) creation of tailor-made plants to supply alternative resources such as starch, fuel and pharmaceuticals to industries.

i) BT cotton: Some strains of *Bacillus thuringiensis* produce proteins that kill insects like lepidocerans (tobacco budworm, armyworm), coleopterans (beetles) and dipterans (flies, mosquitoes). Bt toxins are initially inactive protoxins but after ingestion by the insect they become active due to the alkaline pH of the gut, which solubilises the crystals. The activated toxin binds to the surface of midgut epithelial cells thus creating pores which causes cell swelling and lysis, further leading to death of the insects. Specific Bt toxin genes obtained from *Bacillus thuringiensis* are used in several crop plants like cotton, tobacco etc. The toxin is coded by a gene Cry which is of various types. For example, proteins encoded by the genes *cryIAc* and *cryIIAb* control the cotton bollworm and that of *cryIAb* control corn borer. Bt tobacco was first cultured to kill hornworm (*Manduca sexta).*

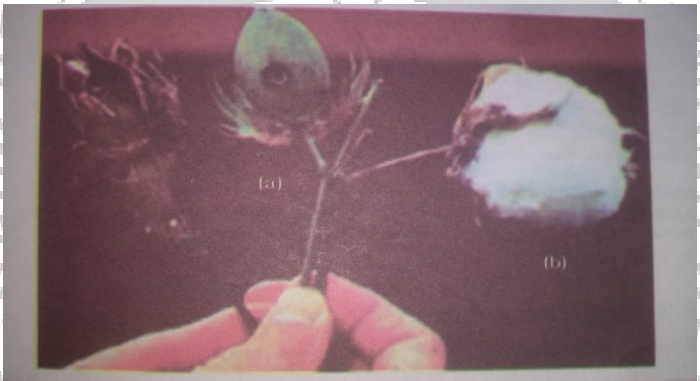

Figure 12.1 Cotton boll: (a) destroyed by bollworms; (b) a fully matur cotton boll

ii) Pest resistant plants: A nematode *Meloidegyne incognitia* infects the roots of tobacco plants which reduces the production of tobacco. It can be prevented by using RNA interference (RNAi) process which is checked by silencing of specific *m*RNA due to a complementary *ds*RNA. *ds*RNA binds and prevents translation of *m*RNA (silencing). By using *Agrobacterium* vectors, nematode-specific genes were introduced into the host plants which produce both sense and anti-sense RNA in the host cells. These two RNAs are complementary to each other and form a double-stranded RNA (*ds*RNA) that initiates RNAi and hence silence the specific *m*RNA of the nematode. The parasite cannot survive in the host, so protects the plants from the pests.

2. **Biotechnological applications in medicine-**

The recombinant DNA technology is used for production of therapeutic drugs which are safe and effective. It avoids unwanted immunological responses, commonly observed with similar products isolated from non-human sources. About 30 recombinant therapeutics have been approved for human use in the world including India.

i) **Genetically engineered insulin**- Insulin contains two short polypeptide chains- Chain A and Chain B linked by disulphide bridges. In mammals, insulin is synthesized as pro-hormone (that needs to be processed to become mature and functional hormone). It contains an extra stretch called C peptide. C peptide is absent in mature insulin and is removed during maturation into insulin. Earlier, insulin was extracted from pancreas of slaughtered pigs and cattle but some patients who were administered with such insulin began developing allergies. In 1983, an American company 'Eli Lilly' began production of insulin (Humulin) by rDNA techniques. It prepared two sequences of DNA corresponding to A and B chains of human insulin and introduced them in plasmids of *E.coli* for production. The A and B chains produced, were separated, extracted and combined, by creating Disulphide bonds to form human insulin.

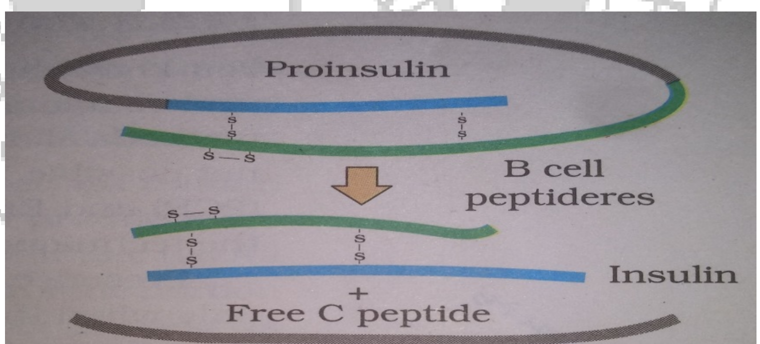

Figure 12.3 Maturation of pro-insulin into insulin after removal of C-peptide (to be simplified)

ii) **Gene therapy-**Gene therapy is a collection of methods that allows correction of gene defects, diagnosed in an embryo or a child. By insertion of normal genes, the defective genes are replaced and non-functional gene is compensated. For the first time in1990, M. Blease and W.F. Andresco of National Institute of

Health, attempted gene therapy on a 4 year old gild with adenosine deaminase (**ADA**) deficiency. **ADA** deficiency is caused due to deletion of gene for ADA. In some cases, it can be cured by bone marrow transplantation and enzyme replacement therapy but it is not fully curative. Lymphocytes from patient's blood were cultured and functional ADA, cDNA was introduced in these lymphocytes using a retrovirus vector. The lymphocytes were then transferred back into the patient's body. Periodic infusion of such genetically engineered lymphocytes needs to be done because these cells are mortal. For permanent cure of ADA deficiency, genes isolated from the bone marrow cells producing ADA , at early embryonic stage can be a possible cure. Other diseases like **cystic fibrosis, haemophilia, cancer, Parkinson's** disease etc., can also be treated by gene therapy.

iii) **Molecular diagnosis-** Early detection of disease if not possible by conventional diagnostic methods, then some other techniques are used for early diagnosis are:

a) Polymerase chain reaction: Low concentration of the pathogen in the body does not allow its detection. The nucleic acid of the pathogen (bacteria/virus) is amplified by PCR for its detection. It is being used for detection of HIV in suspected AIDS patients, COVID-19 (novel corona virus disease reported for the first time in 2019) and genetic mutations in suspected cancer patients.

b) **Recombinant DNA technology**-A single stranded DNA or RNA with known sequence tagged with radioactive molecule is called a probe. In this method, a probe is allowed to hybridise to its complimentary DNA in the clone of cells. the cells are then detected by Autoradiography. The cell with mutated gene will not be observed on the photographic film because the probe was not complementary to the mutated genes.

c) ENZYME LINKED IMMUNOSORBENT ASSAY: This method is based on the antigen-antibody interaction. Either the presences of antigens (proteins, glycoproteins etc.) are detected or antibodies produced against the pathogen are detected.

d) **Stem cell technology**- Stem cells are undifferentiated biological cells. These can differentiate into specialised cells and can divide to produce more stem cells. stem cells are found in

multicellular organisms. Adult stem cells are used in medical therapies, e.g., in bone marrow transplantation. Stem cells can also be taken from umbilical cord blood just after birth and can be stored in stem cell banks for future use if need arises.

3. **Transgenic animals-** Animals whose DNA is manipulated to possess and express an extra (foreign) gene are known as transgenic animals. Transgenic rats, pigs, sheep and cows have been produced. Following are some of the reasons for producing transgenic animals.
   - **i) Study of normal physiology and development-**Transgenic animals are useful in studying gene regulation, their effect on the normal functions of the body and its development. For example, the study of complex growth factors like insulin-like growth factor
   - **ii) Study of diseases-** Study of genes which are responsible for diseases in human and their treatment. Transgenic models have been developed for many human diseases like cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's disease.
   - **iii) Biological products-** Useful biological products can be produced by introducing DNA of interest coding for a particular product, into transgenic animals. For example, human protein (α-1-antitrypsin) for treatment of emphysema. In 1997, the first transgenic cow, Rosie, produced human protein-enriched milk (2.4 g/L). The milk contained the human alpha-lactalbumin and was more nutritionally balanced for human babies than natural cow milk.
   - **iv) Vaccine safety testing-**Transgenic mice were developed to test the safety of vaccines, before being used on humans.
   - **v) Chemical safety testing-**transgenic animals are made to carry genes, which make them more sensitive to the toxic substances of non-transgenic animals. On exposing to the toxic substances, their effects can be studied in lesser time period.

4. **<u>Ethical issues</u>**-Genetic modification of organisms show unpredictable results when such organisms are introduced into the ecosystem. The modification and use of living organisms for public services (such as food and medicine sources) create problems with patents granted. Government of India formed the organisations like GEAC (Genetic engineering approval committee) to decide the validity and safety of GM organisms for public safety. Angered public is questioning that certain companies granted patents for products and technologies which are

grown, identified and used by farmers and indigenous people related to a specific region/country. Rice is being used since thousands of years in Asia's agricultural history, of which 200,000 varieties are in India alone. Basmati is unique for its aroma and flavour, and 27 varieties are cultivated in India. In 1997, an American company got patent rights for Basmati rice through US Patent and Trademark Office, and was allowed to sell a "new' variety in US and abroad. This new variety of Basmati rice was derived from Indian farmer's varieties. Besides Basmati rice, now attempts are in progress for turmeric and neem. Our rich legacy will be reduced by other countries/individuals, if we do not pay attention or counter these patent applications.

5. **Biopiracy** – Biopiracy is defined as the use of bioresources by multinational companies and other organisations, without proper authorisation from the countries and concerned people, without proper compensation. Generally, financially rich nations are poor in biodiversity and traditional knowledge, while developing and under-developed counties are rich in biodiversity and traditional knowledge, related to bioresources. Traditional knowledge related to bioresources can be exploited to develop modern applications and are used to save time, efforts and expenditure during commercialisation. Some nations are developing laws, to prevent such unauthorised exploitation of their bioresources and traditional knowledge. To check these problems, Indian parliament has recently cleared the second amendment on the Indian Patents Bill that takes such issues into consideration.
6. **Patent**-It is a set of exclusive rights granted by a state (national government) to an inventor or other assignee for a limited period of time in exchange for a public disclosure of an invention. Three patents' safety criteria are novelty, non-obviousness and utility.