CHAPTER – 12

BIOTECHNOLOGY AND ITS APPLICATION

Introduction:

Biotechnology mainly deals with industrial scale production of biopharmaceuticals and biological using genetically modified microbes, fungi, plants and animals.

The applications of biotechnology include (i) therapeutics (ii) diagnostics (iii) genetically modified crops for agriculture (iv) Proceeded food (v) bioremediation (vi) Waste treatment (vii) Energy production.

Research Areas of Biotechnology:

Following are three research areas of biotechnology:

(i)Catalyst: Providing the best catalyst in the form of improved organism; generally a microbe or pure enzyme.

(ii)Optimum conditions: Creating optimal conditions through engineering for a catalyst to act.

(iii)Downstream Processing: Downstream Processing technologies to purify the protein or organic compound.

Biotechnological applications in agriculture:

- **Agrochemical based agriculture**: The Green Revolution succeeded in increasing the yield of crops mainly due to
  (i) Use of improved varieties of crops and
  (ii) Use of agrochemicals (fertilizers and pesticides).
- **Organic Agriculture or Organic Farming**: In organic farming, framers use manures, biofertlizers, biopesticides and biocontrols to increase the crop production instead of using artificial fertilizers and pesticides.
- **Genetically Engineered Crop-based Agriculture:** The organic farming cannot increase the yield of crop to appreciable degree. The solution of this problem is use of genetically modified crops. Plants, bacteria, fungi and animals whose genes have been changed by manipulations are called Genetically Modified Organisms (GMOs)
- Crops in which foreign genes have been introduced through genetic engineering are called genetically modified crops or GM Crops.

GMO possess genetic modification like:

(i) made crops more tolerant to abiotic stresses (cold,drought,salt,heat).

(ii) reduced reliance on chemical pesticides (pest-resistant crops).

(iii) helped to reduce post harvest losses.

(iv) Increased efficiency of mineral usage by plants (this prevents early exhaustion of fertility of soil).

(v) Enhanced nutritional value of food, e.g., golden rice, i.e., Vitamin 'A' enriched rice.

Resistance plants: BT Cotton:

- The soil bacterium ***Bacillusthuringiensis*** produces crystal proteins called Cry proteins that are toxic larvae of insects like tobacco budworm, armyworm, beetles and mosquitoes.
- The Cry proteins exit as inactive protoxins and get converted into active toxin when ingested by the insect, as the alkaline ^{H}p of the gut solubilises the crystals.
- The activated toxin binds to the surface of epithelial cells of mid gut and creates pores.
- This causes swelling and lysis of cells leading to the death of the insect (larva).
- The genes (cry genes) encoding this protein are isolated from the bacterium and incorporated into several crop plants like cotton, tomato, corn, rice, soybean etc.
- The proteins encoded by the following cry genes control the pest given against them:
- Cry I Ac and cry II Ab control cotton bollworms.
- Cry I Ab controls corn borer.

Pest resistance plants: RNA interference:

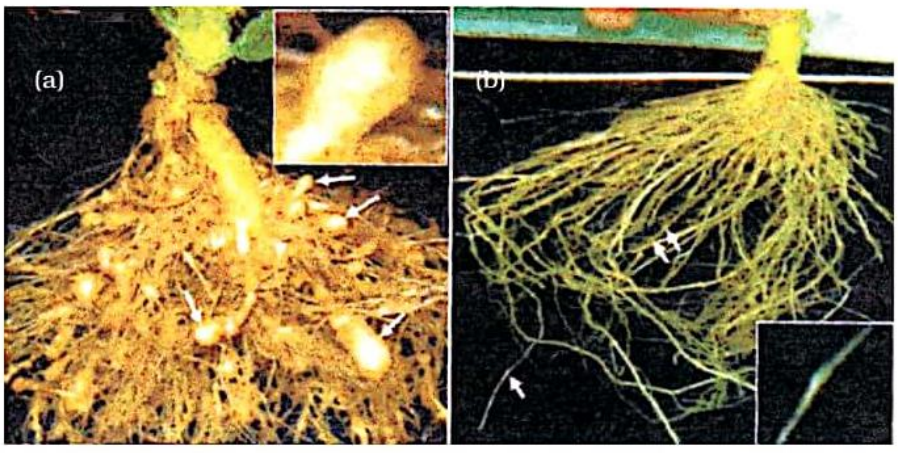

Figure 12.2 Host plant-generated dsRNA triggers protection against nematode infestation: (a) Roots of a typical control plants; (b) transgenic plant roots 5 days after deliberate infection of nematode but protected through novel mechanism.

- Many nematodes live in plants and animals including human beings. A nematode ***Meloidogyne incognitia*** infects the roots of tobacco plants and causes a great reduction in yield.
- A novel strategy was coined by **Fire and Mello** in 1998 to prevent infestation that was based on the process of **RNA interference (RNAi).**
- RNAi takes place in all eukaryotic organisms as a method of cellular defence and this method involves silencing of a specific mRNA.
- The source of this complementary RNA could be from an infection by viruses having RNA genomes or mobile genetic elements (transposons) that replicate via an **RNA intermediate**.
- Using ***Agrobacterium*** vectors, nematode specific genes were introduced into the host plant. The introduction of DNA was such that it produced **both sense and anti-senseRNA** in the host cells.
- These two RNA's being complementary to each other formed a double stranded (**dsRNA**) that initiated RNAi and thus, **silenced the specific mRNA of the nematode.**
- The consequence was that the parasite could not survived in a transgenic host expressing specific interfering RNA and thus the transgenic plant protect itself from the parasite.

Introduction to biotechnological application in medicines:

- The r-DNA technological processes have made great impact in the area of health care by mass production of safe and more effective therapeutic drugs. Further the recombinant therapeutics do not induce unwanted immunological response
- Now about **30 recombinant therapeutics** have been approved for human use all over the world. In **India 12** of these are presently being marketed.

Genetically engineered Insulin:

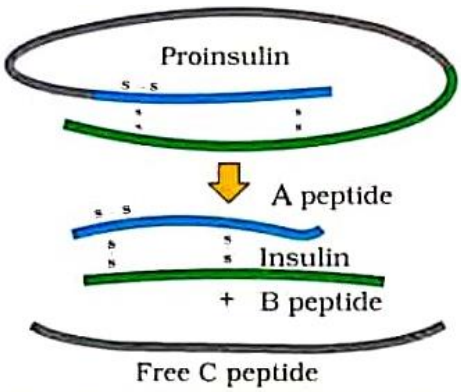

Figure 12.3 Maturation of pro-insulin into insulin (simplified)

- The adult-onset Diabetes is caused due to failure of the islets of pancreas to secrete a hormone named as insulin. It is produced from the beta cells of islets of pancreas.
- Earlier, insulin for curing diabetes used to be extracted from pancreas of **slaughtered pigs and cattle**. This insulin is slightly different from human insulin. So some patients develop **allergy and other types of reaction** to the foreign substance.
- **Human insulin (hormone)** is made up of 51 amino acids arranged in two polypeptide chains: **chain A and chain B**. Both the chains are connected by **2 disulphide bridges**.
- The hormone develops from a storage product called **pro-insulin** (Pro-hormone). This pro-insulin has three chains**: A, B & C**. This **extra stretch of C** chain is **removed during maturation** and the mature insulin is formed.
- In 1983, **Eli Lilly**, an American company, prepared two DNA sequences coding for chain A and B of human insulin and introduced them into the plasmids of ***E.coli*** to produce insulin. Both the chains were produced separately.
- Then both the **chains were extracted and combined by disulphide linkage** to form human insulin.

Recombinant Vaccine:

The recombinant vaccine is a vaccine produced through rDNA technology. This involves inserting the DNA encoding an antigen that stimulates an immune response into bacterial or mammalian cells, expressing the antigens in these cells and then purifying it from them.

Vaccine for hepatitis B virus is being produced from transgenic yeast.

Vaccines against influenza virus, herpes virus and rabies virus have also been developed by rDNA technology.

Gene therapy:

- Gene therapy is a technique of genetic engineering to replace a faulty gene by normal healthy functional gene. It is a collection of methods which allows **correction of genetic defect**. It involves the delivery of normal gene into the individual or embryo to take over the function of and compensate for the non-functional gene.
- It can be conducted in two different ways: **Somatic cell gene therapy** and **germ line gene therapy**. Only introduction of new genes in somatic cells is allowed at present. Genetic modification in the germ cells of the progeny is not permissible.
- The **first clinical gene therapy was given in 1990 to a 4-year old girl** with ADA deficiency.

ADA deficiency:

- Adenosine deaminase enzyme is very crucial for human immune system. Defect/deletion in the gene for enzyme **adenosine deaminase causes ADA deficiency**, which may lead to severe combined immune deficiency **(SCID).**

Treatment of ADA deficiency:

- It can be treated by **enzyme replacement therapy** in some children. In this method the **functional ADA enzyme** is given to a patient by injection. This method is not a permanent cure as periodical infusion is required.
- It also can be treated by **bone marrow transplantation**. The matching and transplant of matched bone marrow from donor to a patient is daunting task. Hence this approach is not completely curative.
- As a first step towards **gene therapy lymphocytes** (WBC) are extracted from the patient and are grown in a culture medium outside the body.
- A **functional ADA cDNA** is then introduced by a **retroviral vector** into the lymphocytes, which are subsequently returned to the patient. However as these **cells are not immortal, the patient requires periodical infusion** of such genetically engineered lymphocytes.
- However, if the gene isolated from bone marrow cells producing ADA is introduced into cells at early embryonic stages, it could be **a permanent cure.**

Molecular diagnosis by biotechnology:

- It is well known that an early diagnosis is very important for the effective treatment of disease. Using conventional methods of diagnosis (serum and urine dialysis), early detection is not possible.
- So rDNA techniques like **PCR, ELISA, molecular probing** serve the purpose of early diagnosis.
- Presence of pathogen (bacteria, viruses) is usually suspected only when the pathogen has produced disease symptoms.
- By this time the number of pathogen is already very high in the body, but **little concentration of virus or bacteria can be detected** by multiplication of their nucleic acid by **PCR**. PCR can detect very low amount of DNA.
- It is used to detect HIV in suspected **AIDS**patients and also the **cancer** patients.
- The molecular probes are very effective in diagnosis of genetic disorders. A **single stranded DNA or RNA joined with radioactive molecule is known as probe.**
- It is allowed to **hybridize to its complementary DNA** in a clone of cells followed by detection using autoradiography. The clone having the mutant gene will hence not appear on the photographic film as the probe will not have complementarity with the mutated gene.
- **ELISA** is based on the principle of **antigen-antibody** interaction. It can detect small amount of protein (antigen/antibody) with the help of enzyme.
- Infection by pathogen can be **detected by the presence of antigens such as proteins, glycoproteins** etc. or by detecting the antibodies synthesized against the pathogens.

Stem cell technology:

- Stem cells are **undifferentiated biological cells**. These can **differentiate into specialized cells, can divide to produce more stem cells** and even give rise to different cells/tissues.
- Stem cells are found in multicellular organisms.
- Adult stem cells are used in medical therapies, for example, in **bone marrow transplantation.**
- Stem cells can also be taken from umbilical cord blood just after birth and can also preserved for future use.
- Stem cells can be useful to treat **diabetes, heart disease, spinal cord injury, cystic fibrosis, cancer, rheumatoid arthiritis,** etc.
- Animal whose DNA is manipulated to possess and express an extra (foreign) gene are known as transgenic animals. Transgenic rats, rabbits, pigs, sheep and cows have been produced. Although over 95% of all existing transgenic animals are mice.
- The followings are the common reasons for developing transgenic animals:

Study of normal physiology and development:

- It is very useful to study gene regulation, their effect on the normal functions of the body and its development in a transgenic.
- For example: study of complex growth factors like **insulin like growth factor**.

Study of diseases:

- Study of genes which are responsible for diseases in human and their treatment is possible with the help of transgenic.
- Transgenic models have been developed for many human diseases like **cancer, cystic fibrosis, rheumatoid arthiritis and Alzheimer's disease.**

Biological products:

- Useful biological products can be produced by introducing, into transgenic animals, the portion of DNA which codes for a particular product.
- For example: human protein **(alpha-1-antitrypsin**) is used to treat **emphysema**. Similar attempts are being made for treatment of phenylketonuria (PKU) and cystic fibrosis.
- In 1997, the first transgenic cow, **Rosie,** produced human protein –enriched milk (2.4g/L).
- The milk contained the **human alpha-lactalbumin** and was more nutritionally balanced for human babies than natural cow milk.

Vaccine safety:

- Transgenic mice are developed to test safety of vaccines, before being used on human. The transgenic animals are much more sensitive than normal organisms.
- For example: **polio vaccine**.

Chemical safety testing:

- Transgenic animals are made to carry genes, which make them more **sensitive to the toxic substances** than non-transgenic animals.
- On exposing to the toxic substances, their effects are studied in less time.

Ethical issues:

- Genetic modification of organisms show unpredictable results when such organisms when they are introduced into ecosystem.
- The modification and use of living organisms for public services creates problem with patents granted.
- Government of India has formed the organization like **GEAC (genetic engineering approval committee)** to decide the validity and safety of GM organisms for public safety.

Biopiracy:

- Biopiracy is defined as the use of bio resources by multinational companies and other organizations without proper authorization from the countries and concerned people, without compensatory payment.
- Generally, financially rich nations are poor in bio diversity and traditional knowledge, while developing and under developed nations are rich in bio diversity and traditional knowledge.
- So the developed countries try to exploit those skills, knowledge and bio resources to save their time, efforts and expenditure without compensation.
- This biopiracy can be studied:
- Rice is being used since thousands of years in Asia's agriculture history, of which 200,000 varieties are in India alone.
- Basmati is unique for its aroma and flavor, whose 27 varieties are cultivated in India.
- In 1997, an American company got patent rights for Basmati rice through the US Patent and Trademark Office, and was allowed to sell a 'new variety' in US and abroad. That Basmati was derived from Indian farmer's variety.
- Indian Basmati was crossed with semi-dwarf varieties and claimed as an invention or a novelity.

BioPatent:

- A patent is a right granted by a government to an inventor to prevent others from commercial use of his/her invention. When patents are granted for biological entities and for products derived from them, these patents are called biopatents.
- To check such problems, Indian Parliament has recently cleared the **second amendment** of the **Indian Patent Bill** that takes such issues into consideration.

Controversies in India regarding patent and biopiracy:

Turmeric: In May 1955, patent was granted by US patent office to University of Mississippi Medical Centre for 'Use of Turmeric in wound healing'. The patent was challenged by Indian scientist and it was established that use of turmeric as healing agent was known in India for centuries.

Neem: In 1996 an Indian scientist challenged the patent granted to the European patent office and established that the fungicidal uses of neem oil mentioned in ancient Indian texts.

Basmati Rice: In 1997, Texas company was given the patent on Basmati rice lines and grains. But later it lost most of the claims of the patent.

IMPORTANT TERMS

Genetically modified organism: Those organisms, whose genes have been altered by manipulation, are called genetically modified organisms or transgenic organisms.

Bt cotton: Cotton plants with Bt gene thereby made resistance to bollworm insects.

RNAi: The process which interferes with the translation of mRNA of the parasite in the host plant.

cDNA: The DNA which is made complementary to the mRNA.

ELISA: Enzyme linked immune sorbent assay. This is a test based on antigen-antibody interaction.

Indian patents Bill: A bill passed by the Indian parliament which takes issues such as terms for patent, research and development initiatives etc.

Gene therapy: It is a collection of methods that allows correction of a gene defect.

Biopiracy: It refers to the use of bio resources by multinational companies and other organizations without proper authorization from the countries and people concerned.

GEAC: Genetically engineering approval committee.

Bt cotton: cotton plant with Bt gene.

Cry gene: gene coding for insecticidal protein.

C peptide: the peptide which is a part of proinsulin and removed before maturation of insulin.

Transgenic animal: the animals with manipulated genes.

cDNA: DNA which is made complimentary to mRNA.

Agrobacterium vectors: the bacteria *Argobacterium tumifaciens* has the natural ability to transfer genes to plant cells.

Meloidegyne incognitia : it is a nematode that affects roots of tobacco plants and reduce their yield.